SPATIALLY-ENCODED ANALYTE DETECTION

Publication number: JP2004500549T
Publication date: 2004-01-08
Inventor:
Applicant:
Classification:
- international: ***G01N33/53; B01L3/00; C12M1/00; C12Q1/68; G01N27/30; G01N27/416; G01N27/447; G01N27/48; G01N33/543; G01N33/566;*** **G01N33/53; B01L3/00; C12M1/00; C12Q1/68; G01N27/30; G01N27/416; G01N27/447; G01N27/48; G01N33/543; G01N33/566;** (IPC1-7): G01N27/447; C12M1/00; C12Q1/68; G01N27/30; G01N27/416; G01N27/48; G01N33/53; G01N33/566
- European: B01L3/00C6M; C12Q1/68B2H; G01N33/543K
Application number: JP20010512919T 20000717
Priority number(s): US19990358204 19990721; WO2000US19502 20000717

Also published as:
WO0107653 (A1)
EP1196636 (A1)
US6294392 (B1)
US2002076714 (A1)
EP1196636 (A0)
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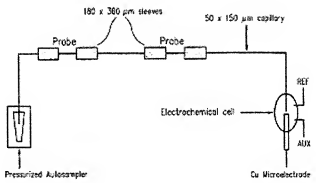
Abstract not available for JP2004500549T
Abstract of corresponding document: **WO0107653**

A flow-through microchannel (e.g. capillary) biosensor is described for the detection of multiple, different analytes (e.g. nucleic acids, proteins, sugars, etc.) targets in a sample by binding them to "complementary" binding partners (e.g. complementary nucleic acids, ligands, antibodies, etc.). The binding partners are immobilized in different sections of a microchannel (e.g. a fused silica capillary). After fabrication of the biosensor, a sample is flushed through the capillary, and any target analyte(s) contained within the sample are bound to the immobilized binding partner(s) on the microchannel wall forming bound complexes. Finally, the bound complexes are simultaneously denatured along the entire length of the capillary and flushed out past a detector poised downstream, and the analyte concentration is measured (e.g., using sinusoidal voltammetry). Direct electrochemical detection of underivatized DNA is accomplished by oxidizing its sugar backbone and the amine containing nucleobase at the copper electrode. The elution time of the desorbed target DNA(s) is used for the sequence identification of the target. Multiple genetic sequences can be diagnosed by using a single biosensor in this manner. The sensor is highly specific due to hybridization chemistry, and extremely sensitive due to electromechanical detection.



Family list
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1 **Spatially-encoded analyte detection**
Inventor: KUHR WERNER G **Applicant:** UNIV CALIFORNIA
EC: B01L3/00C6M; C12Q1/68B2H; (+1) **IPC:** ***G01N33/53; B01L3/00; C12M1/00*** (+20)
Publication info: **AU780575B B2** - 2005-04-07
AU780575C C - 2006-03-30

2 **Spatially-encoded analyte detection**
Inventor: KUHR WERNER G **Applicant:** UNIV CALIFORNIA
EC: B01L3/00C6M; C12Q1/68B2H; (+1) **IPC:** ***G01N33/53; B01L3/00; C12M1/00*** (+18)
Publication info: **AU6107900 A** - 2001-02-13

3 **SPATIALLY-ENCODED ANALYTE DETECTION**
Inventor: KUHR WERNER G (US); BRAZILL SARA ANN (US); (+1) **Applicant:** UNIV CALIFORNIA (US)
EC: B01L3/00C6M; C12Q1/68B2H; (+1) **IPC:** ***G01N33/53; B01L3/00; C12M1/00*** (+18)
Publication info: **CA2375606 A1** - 2001-02-01

4 **SPATIALLY-ENCODED ANALYTE DETECTION**
Inventor: KUHR WERNER G (US) **Applicant:** UNIV CALIFORNIA (US)
EC: B01L3/00C6M; C12Q1/68B2H; (+1) **IPC:** ***G01N33/53; B01L3/00; C12M1/00*** (+18)
Publication info: **EP1196636 A1** - 2002-04-17
EP1196636 A4 - 2004-12-15

5 **SPATIALLY-ENCODED ANALYTE DETECTION**
Inventor: **Applicant:**
EC: B01L3/00C6M; C12Q1/68B2H; (+1) **IPC:** ***G01N33/53; B01L3/00; C12M1/00*** (+25)
Publication info: **JP2004500549T T** - 2004-01-08

6 **Spatially-encoded analyte detection**
Inventor: KUHR WERNER G (US); SINGHAL PANKAJ (US); (+1) **Applicant:** UNIV CALIFORNIA (US)
EC: B01L3/00C6M; C12Q1/68B2H; (+1) **IPC:** ***G01N33/53; B01L3/00; C12M1/00*** (+19)
Publication info: **US6294392 B1** - 2001-09-25

7 **Spatially-encoded analyte detection**
Inventor: KUHR WERNER G (US); SINGHAL PANKAJ (US); (+1) **Applicant:** UNIV CALIFORNIA (US)
EC: B01L3/00C6M; C12Q1/68B2H; (+1) **IPC:** ***G01N33/53; B01L3/00; C12M1/00*** (+21)
Publication info: **US2002076714 A1** - 2002-06-20

8 **SPATIALLY-ENCODED ANALYTE DETECTION**
Inventor: KUHR WERNER G **Applicant:** UNIV CALIFORNIA (US)
EC: B01L3/00C6M; C12Q1/68B2H; (+1) **IPC:** ***G01N33/53; B01L3/00; C12M1/00*** (+18)
Publication info: **WO0107653 A1** - 2001-02-01

Data supplied from the ***esp@cenet*** database - Worldwide

(19)日本国特許庁(JP)　(12)公表特許公報(A)　(11)特許出願公表番号
特表2004-500549
(P2004-500549A)
(43)公表日　平成16年1月8日(2004.1.8)

(51)Int.Cl.[7]	F I			テーマコード（参考）
G01N 27/447	G01N	27/26	331E	4B029
C12M 1/00	C12M	1/00	A	4B063
C12Q 1/68	C12Q	1/68	A	
G01N 27/30	G01N	27/30	B	
G01N 27/416	G01N	27/30	F	

審査請求 未請求　予備審査請求 有　（全 86 頁）　最終頁に続く

(21)出願番号　特願2001-512910(P2001-512910)
(86)(22)出願日　平成12年7月17日(2000.7.17)
(85)翻訳文提出日　平成14年1月17日(2002.1.17)
(86)国際出願番号　PCT/US2000/019502
(87)国際公開番号　WO2001/007653
(87)国際公開日　平成13年2月1日(2001.2.1)
(31)優先権主張番号　09/358,204
(32)優先日　平成11年7月21日(1999.7.21)
(33)優先権主張国　米国(US)
(81)指定国　EP(AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE),AU,CA,JP,KR,NO

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Fターム(参考)　4B029 AA07 BB15 CC01 FA02 FA10 FA15 GB01 GB06

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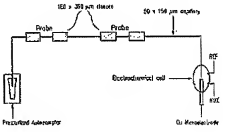
(54)【発明の名称】空間的にコード化された分析物の検出

(57)【要約】
サンプル中の複数の異なる分析物（例えば、核酸、タンパク質、糖質など）種の、それらを「相補的な」結合相手（例えば、相補的核酸、リガンド、抗体など）と結合させることによる検出のためのフロースルーマイクロチャネル（例えばキャピラリー）バイオセンサが記載される。結合相手はマイクロチャネル（例えば、溶融シリカキャピラリー）の異なるセクションに固定化される。バイオセンサの操作の後、サンプルがキャピラリー内に流され、サンプル内に含まれるいずれかの種の分析物はマイクロチャネル壁の固定化された結合相手と結合し、結合複合体を形成する。最後に、結合複合体はキャピラリーの全長に沿って同時に変性され、下流に配置された検出器を通過して流され、分析物濃度が（例えば、シンソイドボルタンメトリーを用いて）測定される。



【特許請求の範囲】
【請求項１】
サンプル中の２つ以上の標的分析物を検出するための方法であって、該方法は、以下の工程：
ｉ）該２つ以上の分析物の各々に対する結合対がその中に固定されているチャネルを提供する工程であって、ここで該２つ以上の分析物の各々に対する結合対は、該チャネルの異なる領域に位置づけられ、そして該チャネルは、十分に小さい断面積を有し、その結果、該２つ以上の結合対から、該チャネルを通って流れている流体に分析物が放出される場合に、該分析物が該結合対から下流にある該チャネルの検出点に到達するまで、該分析物が空間的に隔離されたままである、工程；
ｉｉ）サンプルを含む流体を、該流体中に存在する該標的分析物がそれらのそれぞれの結合対と結合するような条件下で、該チャネルを通して流す工程であって、それによって該分析物を該チャネルに沿って空間的にコード化する、工程、
ｉｉｉ）該分析物を、該結合対から、該チャネルに沿って流れている流体へ放出する工程；および
ｉｖ）該結合対から下流にある該チャネルに沿った位置で、該分析物を検出する工程、
を包含する、方法。
【請求項２】
前記分析物が標識されていない、請求項１に記載の方法。
【請求項３】
前記チャネルが毛細管である、請求項１に記載の方法。
【請求項４】
前記毛細管がキャピラリー電気泳動管である、請求項３に記載の方法。
【請求項５】
前記チャネルが、表面にエッチングされたチャネルである、請求項１に記載の方法。
【請求項６】
前記チャネルが、ガラス表面にエッチングされたチャネルである、請求項５に記載の方法。
【請求項７】
前記チャネルが成型されている、請求項１に記載の方法。
【請求項８】
前記チャネルが、ポリマー材料で成型されている、請求項７に記載の方法。
【請求項９】
前記チャネルが、約１未満のレイノルズ数（Ｒｅ）を提供する断面積を有する、請求項１に記載の方法。
【請求項１０】
前記チャネルが、約１００μｍ未満の断面直径を有する、請求項１に記載の方法。
【請求項１１】
前記２つ以上の標的分析物が、少なくとも３つの異なる分析物を含む、請求項１に記載の方法。
【請求項１２】
前記結合対が、抗体、結合タンパク質、および核酸からなる群より選択される、請求項１に記載の方法。
【請求項１３】
前記結合対が核酸である、請求項１２に記載の方法。
【請求項１４】
前記流体を流す工程が、圧力差によって誘導される流体流れである、請求項１に記載の方法。
【請求項１５】
前記流体を流す工程が、電気浸透流体流れである、請求項１に記載の方法。

【請求項１６】
前記流体が、血液、血漿、血清、尿、口腔液、脳脊髄液、およびリンパからなる群より選択されるサンプルを含む、請求項１に記載の方法。
【請求項１７】
前記検出工程が、吸光分光法を含む、請求項１に記載の方法。
【請求項１８】
前記検出工程が、シヌソイドボルタンメトリーを含む、請求項１に記載の方法。
【請求項１９】
前記分析物が核酸であり、前記検出工程が、１×１０$^{-9}$Ｍ未満の濃度で標的分析物を検出する、請求項１に記載の方法。
【請求項２０】
サンプル中の２つ以上の分析物を検出するためのデバイスであって、該デバイスは、以下：
該２つ以上の分析物の各々に対する結合相手がその中に固定されているチャネルであって、ここで該２つ以上の分析物の各々に対する該結合相手は、該チャネルの異なる領域に位置づけられ、そして該チャネルは、十分に小さい断面積を有し、その結果、該２つ以上の結合相手から、該チャネルを通って流れている流体に分析物が放出される場合に、該分析物が該結合相手より下流にある該チャネルに沿った検出点に到達するまで、該分析物が空間的に隔離されたままである、チャネル；および
該分析物を、該チャネル内の該検出点で検出する、検出器、
を備える、デバイス。
【請求項２１】
前記チャネルが毛細管である、請求項２０に記載のデバイス。
【請求項２２】
前記毛細管が、キャピラリー電気泳動管である、請求項２１に記載のデバイス。
【請求項２３】
前記チャネルが、表面にエッチングされたチャネルである、請求項２０に記載のデバイス。
【請求項２４】
前記チャネルが、ガラス表面にエッチングされたチャネルである、請求項２３に記載のデバイス。
【請求項２５】
前記チャネルが、約１未満のレイノルズ数（Ｒｅ）を提供する断面積を有する、請求項２０に記載のデバイス。
【請求項２６】
前記チャネルが、約１００μｍ未満の断面直径を有する、請求項２０に記載のデバイス。
【請求項２７】
前記２つ以上の標的分析物が、少なくとも３つの異なる分析物を含む、請求項２０に記載のデバイス。
【請求項２８】
前記結合相手が、抗体、結合タンパク質、および核酸からなる群より選択される、請求項２０に記載のデバイス。
【請求項２９】
前記結合相手が核酸である、請求項２８に記載のデバイス。
【請求項３０】
前記検出器が、吸光分析計を備える、請求項２０に記載のデバイス。
【請求項３１】
前記検出器が、シヌソイド電圧電流計を備える、請求項２０に記載のデバイス。
【請求項３２】
流体中の２つ以上の標的分析物の検出のためのキットであって、該キットは、該２つ以上

の分析物の各々に対する結合相手がその中に固定されているチャネルを備え、ここで該２つ以上の分析物の各々に対する該結合相手は、該チャネルの異なる領域に位置づけられ、そして該チャネルは、十分に小さい断面積を有し、その結果、該２つ以上の結合相手から、該チャネルを通って流れている流体に分析物が放出される場合に、該分析物が該結合相手より下流にある該チャネルに沿った検出点に到達するまで、該分析物が空間的に隔離されたままである、
キット。
【請求項３３】
前記キットが、複数の前記チャネルを備える、請求項３２に記載のキット。
【請求項３４】
前記複数のチャネルを含む前記チャネルの各々が、結合相手の固有の収集を有する、請求項３３に記載のキット。
【請求項３５】
前記チャネルが毛細管である、請求項３３に記載のキット。
【請求項３６】
前記毛細管が、キャピラリー電気泳動管である、請求項３５に記載のキット。
【請求項３７】
前記チャネルが、表面にエッチングされたチャネルである、請求項３３に記載のキット。
【請求項３８】
前記チャネルが、ガラス表面にエッチングされたチャネルである、請求項３７に記載のキット。
【請求項３９】
前記チャネルが、約１未満のレイノルズ数（Ｒｅ）を提供する断面積を有する、請求項３３に記載のキット。
【請求項４０】
前記チャネルが、約１００μｍ未満の断面直径を有する、請求項３３に記載のキット。
【請求項４１】
前記チャネルが、少なくとも３つの異なる種の結合相手を含む、請求項３３に記載のキット。
【請求項４２】
前記結合相手が、抗体、結合タンパク質、および核酸からなる群より選択される、請求項３３に記載のキット。
【請求項４３】
前記結合相手が核酸である、請求項４２に記載のキット。
【発明の詳細な説明】
【０００１】
（関連出願の相互参照）
本願は、すべての目的で本明細書中にその全体が参考として援用される、１９９９年７月２１日出願の米国特許出願第０９／３５８，２０４号の優先権を主張するものである。
【０００２】
（連邦後援研究開発の下で行われる発明の権利に関する申し立て）
本研究は、国立予防衛生研究所（ＧＭ４４１１２－０１Ａ１）およびＵＣ　ＢｉｏＳＴＡＲプロジェクトによって支援された。アメリカ合衆国政府は、本発明における一定の権利を有し得る。
【０００３】
（発明の分野）
本発明は、診断の分野に関する。詳細には、本発明は、複数の分析物の迅速な検出および／または定量を可能にしながらも、標識または標識づけ工程の使用を必要としないデバイスおよび方法を提供する。
【０００４】

(発明の背景)
イムノアッセイおよび核酸ハイブリダイゼーション化学は、遺伝的欠陥を検出する、疾病診断を実行する、および予後の評価を実行するという目標に向けて急速に開発されている（Sosnowskiら（1997）Proc. Natl. Acad. Sci. USA, 94：1119－1123）。抗体、核酸結合タンパク質、レセプターリガンドおよび核酸は、非常に特異的にかつ高効率で、適切な条件下で各自の同系の「結合相手」と結合することが知られている。この現象は、病原体（例えばHIV）、病理学的状態（例えば、癌、肝臓病、腎臓病、変性関節疾患など）、薬物乱用（例えば、コチニンなどといった生成物の検出）などの認識および診断に頻繁に使用されている。

【0005】
多数の疾病マーカーおよび病原体マーカー（例えば、タンパク質および／または核酸）が周知であり、完全に特徴づけられてきた。従って、そのようなマーカーと特異的に結合する結合相手（例えば、核酸、抗体など）は、合成および／または単離され、疾病状態または病原体の認識のためのマーカーとして使用することができる（Landegrenら（1988）Science, 242：229、Mikkelson（1996）Electroanalysis, 8：15－19）。様々なアッセイが、そうした方法を用いて微生物学実験室または病理学実験室において日常的に行われている。

【0006】
核酸ハイブダイゼーション、抗体結合反応、タンパク質結合反応およびレクチン結合反応は一般に、分子に（例えばDNAの二重らせんに）挿入するかまたは、標的もしくはプローブ分子のどちらか一方に共有結合で固定されるかのどちらかである標識の使用によって検出される（例えば、Sosnowskiら（1997）Proc. Natl. Acad. Sci. USA, 94：1119－1123、LePecqおよびPaoletti（1966）Anal. Biochem., 17：100－107、KapuscinskiおよびSkoczylas（1977）Anal. Biochem., 83：252－257参照）。場合によっては、電気化学発光もまた、挿入された電気活性発光マーカーを検出するために使用されている（Pollard－Knightsら（1990）Anal. Biochem., 185：84－89、Pollard－Knightsら（1990）Anal. Biochem., 185：353－358、Tizardら（1990）Proc. Natl. Acad. Sci. USA, 12：4514－4518）。これらの検出戦略の全部は、プローブと標的分子との間の結合反応の前（例えば共有結合標識づけのために）または後（例えば挿入または間接的標識づけのために）のどちらか一方で、標的またはプローブ分子の誘導体化を必要とする。これは汚染問題をもたらす。さらに、複数の分析物が同時に分析される場合、複数の標識が使用されなければならない。さらに、煩雑なサンプル取扱が要求され、それはさらに、汚染のリスクを増大しかつ／または誤った分析につながる。上記および他の問題は、本発明によって克服される。

【0007】
(発明の要約)
本発明は、サンプル中の複数の分析物を検出および／または定量化する新規なデバイスおよび方法を提供する。本発明は、各自の同系の「結合相手」（例えば、核酸、抗体、レクチンなど）と結合した後に、サンプル中の異なる標的分析物（例えば、核酸）を検出するフロースルー微小流体（例えばキャピラリー）バイオセンサを提供する。一般に、各種分析物に特異的な結合相手「プローブ」が、例えば感光性ビオチン／アビジン技術を用いるなどして、キャピラリーチャネルの異なるセクションに固定化される。サンプルがその後キャピラリー内にフラッシュされ、その結果、標的分析物はキャピラリー壁に固定化された結合相手（捕獲剤）と結合し、残りのサンプルはキャピラリーから溶離される。最後に、複合体を形成した（結合した）分析物は、チャネルの全長に沿って放出され、検出器を通過してフラッシュされる。好ましい実施形態において、脱着した標的－分析物は、下流に配備された銅電極においてシヌソイドボルタンメトリーを用いて検出される（Singhalおよび Kuhr（1997）Anal. Chem., 69：3552－3557、

Ｓｉｎｇｈａｌら（１９９７）Ａｎａｌ．Ｃｈｅｍ．，６９：１６６２－１６６８）。標的分析物の溶離から検出までの時間は、個々の分析物の正体を決定するために使用される。同一種の分子の（例えば、すべて核酸）、または異なる種（例えば、タンパク質および核酸）の複数の分析物が、このようにして単一のバイオセンサを用いて診断できる。センサは、特異的な結合相手の使用により高度に特異的であり、電気化学的検出により極めて高感度である。

【０００８】

従って、１実施形態において、本発明は、サンプル中の２以上の分析物を検出するデバイスを提供する。このデバイスは、２以上の分析物の各々の結合相手が固定されているチャネルを含み、ここにおいて、２以上の分析物の各々の結合相手はチャネルの異なる領域に配置されており、チャネルは十分に小さい断面積を有しており、それによって分析物が２以上の結合相手からチャネル内を流れる流体に放出されたときに、分析物は、結合相手から下流のチャネルに沿った検出ポイントに、またはその端に、そして検出ポイントで分析物を検出する検出器に到達するまで、空間的に分離されたままである。

【０００９】

チャネルは、例えば毛細管、キャピラリー電気泳動管、表面にエッチングされたチャネル、表面上にプリントされた疎水剤により形成されたチャネルなど、あらゆる恒直的なチャネルであり得る。チャネルは、分析物が、チャネルにおける検出領域またはチャネル端に到達したときに識別されるように十分に分離され続ける限り、本質的にあらゆる寸法を有することができる。好ましいチャネルは、約１未満のレイノルズ数（Ｒｅ）を与える断面積を有する。好ましいチャネルは、約５００μｍ以下の、より好ましくは約１００μｍ以下の、最も好ましくは約５０μｍ以下の、断面直径または幅を有する。特に好ましいデバイスにおいて、２以上の標的分析物は、少なくとも３、好ましくは少なくとも４、より好ましくは少なくとも５、最も好ましくは少なくとも１０、少なくとも５０、少なくとも１００または少なくとも５００の異なる分析物（および、それゆえその多数の異なる結合相手）を含む。以下に限らないが、抗体、結合タンパク質および核酸を含め、多種多様な結合相手が適切である。同様に、多くの検出器が適切であり、分光光度計（例えば、吸光度分光光度計）および（本質的にあらゆる電流測定および／または ボルタンメトリーおよび／または電位差および／または電量分析検出器を含む）電気分析的検出器が挙げられる。好ましい検出器としては、電圧電流計、特にシヌソイド電圧電流計が挙げられる。

【００１０】

別の実施形態において、本発明は、サンプル中の２以上の標的分析物を検出する方法を提供する。この方法は、本明細書中に記載される検出デバイスを提供する工程；ｉｉ）サンプルを含む流体を、流体に存在する標的分析物がそれぞれ各自の結合相手と結合する条件下でチャネルを通過させ、それにより分析物をチャネルに沿って空間的にコード化する工程；ｉｉｉ）チャネルに沿って通過している流体の流れに分析物を結合相手から放出する工程；ｉｖ）結合相手から下流のチャネルに沿った位置で分析物を検出する工程を包含する。好ましい方法において、分析物は標識づけされない。特に好ましい実施形態において、分析物は標識づけされない。特に好ましいデバイスにおいて、２以上の標的分析物は、少なくとも３、好ましくは少なくとも４、より好ましくは少なくとも５、最も好ましくは少なくとも１０、少なくとも５０、少なくとも１００または少なくとも５００の異なる分析物を含む（そしてそれゆえ、その多数の異なる結合相手が検出デバイスを含むチャネルに存在する）。いくつかの好ましい実施形態において、流体流れは、圧力差および／または電気浸透流によって誘起される。流体流れ。分析物の検出のために好ましい「サンプル」流体としては、血液、血漿、血清、尿、口腔内液、脳脊髄液およびリンパが挙げられる。検出は、分光光度計（例えば、吸光度分光光度法）および（本質的にあらゆる電流測定および／またはボルタンメトリーおよび／または電位差および／または電量分析法を含む）電気分析的方法を含む、多様な方法によることができる。好ましい検出方法は、ボルタンメトリー、特にシヌソイドボルタンメトリーである。特に好ましい方法において、分析物は核酸であり、検出は１×１０－９Ｍ未満の濃度で標的分析物を検出する。

【００１１】
（定義）
用語「ポリペプチド」、「ペプチド」および「タンパク質」は、本明細書中では、アミノ酸残基の重合体を指すために互換可能に使用される。これらの用語は、１つ以上のアミノ酸残基が対応する天然に存在するアミノ酸の人工的な化学的アナログであるアミノ酸重合体だけでなく、天然に存在するアミノ酸重合体にもあてはまる。
【００１２】
用語「抗体」は、本明細書中で使用される通り、インタクトな免疫グロブリン、軽鎖および重鎖の可変領域だけを含んでいるＦｖフラグメント、ジスルフィド結合により結合されたＦｖフラグメント（Ｂｒｉｎｋｍａｎｎら（１９９３）Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ，９０：５４７－５５１）、可変領域と定常領域の部分を含んでいるＦａｂまたは（Ｆａｂ）’２フラグメント、一本鎖抗体などを含む、様々な形態の修飾または改変された抗体を含む（Ｂｉｒｄら（１９８８）Ｓｃｉｅｎｃｅ ２４２：４２４－４２６；Ｈｕｓｔｏｎら（１９８８）Ｐｒｏｃ．Ｎａｔ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ ８５：５８７９－５８８３）。抗体は、動物（特にマウスまたはラット）またはヒト由来であり得るか、またはキメラ（Ｍｏｒｒｉｓｏｎら（１９８４）Ｐｒｏｃ Ｎａｔ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ ８１：６８５１－６８５５）もしくはヒト化（ｊｏｎｅｓら（１９８６）Ｎａｔｕｒｅ ３２１：５２２－５２５；および公開イギリス特許出願＃８７０７５２）であり得る。
【００１３】
用語「結合相手」または「捕獲剤」または「結合ペア」のメンバーは、抗体－抗原、レクチン－炭水化物、核酸－核酸、ビオチン－アビジンなどといった結合複合体を形成するために他の分子と特異的に結合する分子をいう。特に好ましい実施形態において、結合は、非共有結合（例えばイオン、疎水）相互作用によって主として成立する。
【００１４】
用語「特異的に結合する」は、本明細書中で使用されるように、生体分子（例えば、タンパク質、核酸、抗体など）を指す場合に、分子（例えば、タンパク質および他の生物学的製剤）の異種集団における生体分子の存在を決定づける結合反応をいう。従って、指定された条件下で（例えば、抗体の場合におけるイムノアッセイ条件、または核酸の場合におけるストリンジェントハイブリダイゼーション条件）、特定のリガンドまたは抗体は、その特定の「標的」分子と結合し、サンプル中に存在する他の分子と有意な量で結合しない。
【００１５】
用語「チャネル」は、流体の流れを特定の方向に導く経路をいう。チャネルは、底部および側部を有する溝もしくはトレンチ、または完全に包囲された「管」として形成することができる。一部の実施形態では、チャネルは「側部」を有する必要さえない。例えば、疎水性ポリマーを平坦な表面に適用し、それによって狭い（例えば親水性）範囲でその表面での流体の流れを制限および／または誘導することができる。チャネルは好ましくは、結合相手（捕獲）薬剤が固定され得る少なくとも１つの表面を備える。
【００１６】
「標的分析物」は、サンプルにおいて検出および／または定量化されるべきあらゆる単数または複数の分子である。好ましい標的分析物としては、核酸、抗体、タンパク質、糖類などの生体分子が挙げられる。
【００１７】
用語「マイクロチャネル」は本明細書中で、低レイノルズ数操作（Ｒｅ≦１、好ましくはＲｅ≦０．１、より好ましくはＲｅ≦０．０１、最も好ましくはＲｅ≦０．００１）を可能にする寸法を有するチャネルについて使用される。一般に低レイノルズ数操作、流体力学は、慣性力よりもむしろ粘性力によって支配される。
【００１８】
用語毛細管（キャピラリー）は、狭い寸法の管（例えば一般に低Ｒｅの流れを与える）を

いう。開放端毛細管は、水と接触したときに、一般に毛管作用によって水を吸い上げる。毛細管は、以下に限らないが、ガラス、プラスチック、石英、セラミックおよび各種ケイ酸塩を含む、多くの材料で製作することができる。

【００１９】

「キャピラリー電気泳動管」は、キャピラリー電気泳動デバイスにおいて、そのために設計および／または一般に使用される、または使用されるように意図された「毛細管」をいう。

【００２０】

用語「核酸」または「オリゴヌクレオチド」または文法的に同等の語句は本明細書中で、共有結合により一体に結合された少なくとも２個のヌクレオチドをいう。本発明の核酸は好ましくは、一本鎖または二本鎖であり、一般にホスホジエステル結合を含むが、場合によっては以下に概説するように、例えば、ホスホルアミド（Beaucageら（１９９３）Tetrahedron 49（１０）：１９２５）およびその参考文献；Letsinger（１９７０）J. Org. Chem. 35：３８００；Sprinzlら（１９７７）Eur. J. Biochem. 81：５７９；Letsingerら（１９８６）Nucl. Acids Res. 14：３４８７；Sawaiら（１９８４）Chem. Lett. ８０５，Letsingerら（１９８８）J. Am. Chem. Soc. １１０：４４７０；およびPauwelsら（１９８６）Chemica Scripta 26：１４１　９）、ホスホロチオエート（Magら（１９９１）Nucleic Acids Res. １９：１４３７；および米国特許第５，６４４，０４８号）、ホスホロジチオエート（Briuら（１９８９）J. Am. Chem. Soc. １１１　：２３２１）、Ｏ－メチルホスホロアミダイト（O-methylphophoroamidite）結合（Eckstein，Oligonucleotides and Analogues：A Practical Approach，Oxford University Press参照）、ならびにペプチド核酸骨格および結合（Egholm（１９９２）J. Am. Chem. Soc. １１４：１８９５；Meierら（１９９２）Chem. Int. Ed. Engl. ３１：１００８；Nielsen（１９９３）Nature，３６５：５６６；Carlssonら（１９９６）Nature ３８０：２０７参照）を含む、交互骨格を有し得る核酸アナログが含まれる。他の類似の核酸は、陽性骨格（Denpcyら（１９９５）Proc. Natl. Acad. Sci. USA ９２：６０９７）、非イオン性骨格（米国特許第５，３８６，０２３号、同第５，６３７，６８４号、同第５，６０２，２４０号、同第５，２１６，１４１号および同第４，４６９，８６３号；Angew. （１９９１）Chem. Intl. Ed. English ３０：４２３；Letsingerら（１９８８）J. Am. Chem. Soc. １１０：４４７０；Letsingerら（１９９４）Nucleoside & Nucleotide １３：１５９７；第２章および第３章，ASC Symposium Series ５８０，"Carbohydrate Modifications in Antisense Research"，Y. S. SanghuiおよびP. Dan Cook編；Mesmaekerら（１９９４），Bioorganic & Medicinal Chem. Lett. ４：３９５；Jeffsら（１９９４）J. Biomolecular NMR ３４：１７：Tetrahedron Lett. ３７：７４３（１９９６））、ならびに米国特許第５，２３５，０３３号および同第５，０３４，５０６号ならびに前掲書第６章および第７章（ASC Symposium Series ５８０．"Carbohydrate Modifications in Antisense Research"，Y. S. SanghuiおよびP. Dan Cook編）に記載のものを含む、ノンリボース骨格を備えるものを含む。１以上の炭素環式糖類を含有する核酸もまた、核酸の定義の内に含まれる（Jenkinsら（１９９５），Chem. Soc. Rev. １６９－１７６頁参照）。いくつかの核酸アナログがRawls（Rawls，C&E News Jun. ２，１９９７，３５頁）に記載されている。リボースーリン酸塩骨格のこれらの修飾は、標識といった付加的部分の付加を容易に

するか、または生理学的環境における当該分子の安定性および半減期を増大させるために行うことができる。

用語「と特異的にハイブリダイズする」および「特異的ハイブリダイゼーション」および「と選択的にハイブリダイズする」は、本明細書中で使用される通り、ストリンジェント条件の下で特定のヌクレオチド配列に対する選択的な、核酸分子の結合、二重化またはハイブリダイジングをいう。用語「ストリンジェント条件」は、プローブがその標的配列と選択的にハイブリダイズし、そして他の配列とは少ない程度にするかまたはまったくしない条件をいう。核酸ハイブリダイゼーションの状況におけるストリンジェントハイブリダイゼーションおよびストリンジェントハイブリダイゼーション洗浄条件は、配列依存的であり、異なる環境パラメータの下で異なる。核酸ハイブリダイゼーションの包括的な手引きは、例えばTijssen（1993）Laboratory Techniques in Biochemistry and Molecular Biology－Hybridization with Nucleic Acid Probes 第I節，第2章，Overview of principles of hybridization and the strategy of nucleic acid probe assays，Elsevier，N.Y.（Tijssen）に見出される。一般に、高度にストリンジェントなハイブリダイゼーションおよび洗浄条件は、規定のイオン強度およびpHで特定の配列の熱的融点（T_m）よりも約5℃低いものであるように選択される。T_mは、（規定のイオン強度およびpHの下で）標的配列の50％が、完全に合致したプローブとハイブリダイズする温度である。極めてストリンジェントな条件は、特定のプローブについてのT_mと等しいように選択される。サザンブロットまたはノーザンブロットにおいてアレイまたはフィルタに100を超える相補的残基を有する相補的核酸のハイブリダイゼーションのためのストリンジェントハイブリダイゼーション条件の一例は、標準ハイブリダイゼーション溶液を用いて42℃であり（例えばSambrook（1989）Molecular Cloning：A Laboratory Manual（第2版）1－3巻，Cold Spring Harbor Laboratory，Cold Spring Harbor Press，NYおよび以下の詳細な説明を参照）、ハイブリダイゼーションは終夜実行される。高度にストリンジェントな洗浄条件の一例は、約15分間、72℃での0．15M NaClである。ストリンジェント洗浄条件の一例は、SSC緩衝液の種目の場合、15分間、65℃での0．2×SSC洗浄である（例えば前掲Sambrook参照）。たいてい、バックグラウンドプローブ信号を除去するために、高ストリンジェンシー洗浄には低ストリンジェンシー洗浄が先行する。例えば100を超えるヌクレオチドの二重化のための中程度のストリンジェンシー洗浄の一例は、15分間、45℃での1×SSCである。例えば100を超えるヌクレオチドの二重化のための低ストリンジェンシー洗浄の一例は、15分間、40℃での4×～6×SSCである。

【0021】

「空間的分離」は、流体ストリームにおける2以上の種の分子（例えば分析物）の濃度分布の局在化の相違をいう。分析物が空間的に分離されたが（すなわち、フローコード化された）場合、たとえ分析物の全部の信号のタイプが同一であったとしても、目的の個々の分析物の個別の信号を検出することが可能である。従って、分析物の正体は検出の「流路」に沿った位置または時間によって決定することができ、個々の分析物に関係する標識の相違は要求されない。

【0022】

電気分析的方法は、その系に関する情報を取り出すために系または分析物の「電気的」特性（例えば、抵抗、コンダクタンス、キャパシタンス、インピーダンスなど）を利用する方法をいう。電気分析的方法としては、本質的にあらゆる電流測定および／またはボルタンメトリーおよび／または電位差および／または電量分析方法が挙げられる。好ましい電気分析的方法としては、サイクリックボルタンメトリー、交流、直流または回転リングディスクボルタンメトリー、シヌソイドボルタンメトリー、インピーダンス分光法などが挙

げられる。

【００２３】

用語「サイクリックボルタンメトリー」または「経時変化ボルタンメトリー」は、サイクリックボルタンメトリーを指すために互換可能に使用される。用語「シヌソイドボルタンメトリー」は、一般に（例えば、以下に限らないが、方形波、三角波などを含むいずれかの経時変化電圧による）サイクリックボルタンメトリーを指すため、または例えば米国特許第５，６５０，０６１号に記載の通り、サイクリックボルタンメトリーと類似の態様で使用される大振幅正弦波電位波形の使用を指すために使用される。

【００２４】

（詳細な説明）

（Ｉ．複数分析物の効率的検出方法）

本発明は、サンプル中の複数の分析物の迅速な検出および／または定量化のための新規な方法および器械を提供する。好ましい１実施形態において、本発明は、検出が望まれる分析物に特異的な結合相手をその中に固定したチャネルを含む。異なる結合相手がチャネルの異なる領域に配置されるので、分析物が結合された時に、それらはチャネルに沿った各自の位置によって「空間的にコード化される」。結合された分析物は後に結合相手から解放されるか、または、結合相手／分析物複合体はチャネルの壁から、チャネル内を流れる流体中に放出される。チャネルの寸法は、分析物が上記結合相手から下流のチャネルの検出ポイントに到達するまで分析物が空間的に分離され続けるようなものである。

【００２５】

分析物または分析物／結合相手複合体が流れに放出されると、それらは空間的にコード化される。すなわち、ストリームでの相互に対する各自の位置は、それらがチャネル壁に固定されていた時の結合相手の位置に依存する。従って、放出と検出との間の時間差は、出力信号を生成する（または生成しない）特定の分析物を特異的に識別するために使用することができる。

【００２６】

分析物が各々、それらを他の分析物から区別するために標識を使用することなく特異的に識別され得るので、多数かつ冗長なサンプル取扱および標識化工程が排除される。これは多数の操作および汚染問題を取り除く。また、偽陽性の高い発生率につながり得るサンプル汚染のリスクも低減または排除される。

【００２７】

チャネルが使用前に良好に準備でき、各種微小流体構造（例えばチャネル）がサンプル取扱、流体の流れおよび分析物検出を行うデバイスの内外へ交換できることが特筆される。異なるチャネルが異なる集合の分析物に合わせて備えることができ、同一または異なる複数のチャネルが同時に実行され得る。

【００２８】

従って、本発明の方法およびデバイスは、臨床環境における分析物の検出に良好に適する。非誘導体化分析物（例えばＤＮＡ、ｍＲＮＡ、等）を検出する能力は、手順を著しく簡素化し、サンプル汚染および誤った識別の問題の防止を助成する。

【００２９】

特に好ましい１実施形態において、サイクリック（例えばシヌソイド）ボルタンメトリーによる銅電極の使用は、従来の電気化学的測定法が遭遇する問題の多くを克服し、それによって分析物の検出を可能にする。その検出戦略の高感度は、周波数ドメインにおける容量性バックグラウンド電流からのファラデー信号の効果的な減結合に起因している。従って、例えばｓｓＤＮＡやｄｓＤＮＡはピコモル濃度レンジにおいて検出することができ、電気化学的信号は、同じサイズのｓｓＤＮＡに比べて、ＤＮＡ二重らせんの外周の容易にアクセスできる糖類の酸化に起因する。

【００３０】

ただ１個の検出器を使用して複数の標的を検出できるセンサが、製作も容易なより安価かつ小型の検出システムを提供する。

【００３１】
（ＩＩ．システム構成要素）
（Ａ）チャネル）
（１）チャネルのタイプおよび寸法）
チャネルは、チャネルに沿った異なる位置で溶液中の成分間の本質的な混合を伴わずにチャネル内部の物質の通過を可能にする限り、事実上どのようなタイプのチャネルでも本発明の実施に適する。すなわち、好ましいキャピラリーにおいて、チャネルに沿った個別の場所で最初に放出された分析物（または他の検出可能な試薬）は、初期放出ポイントから「下流」の検出ポイントで空間的に分離され続ける。空間的分離とは、たとえ分析物の全部に関する信号のタイプが同一であったとしても、目的の各分析物の個別の信号を検出できる能力をいう。従って、分析物の正体は「流路」に沿った位置または検出の時間によって決定することができ、個々の分析物に関係する標識の相違は要求されない。
【００３２】
しかし、空間的分離は分析物相互の完全な分離を要求するものではない。反対に、相当の重なり合いが存在することができ、ピーク濃度が検出でき、関係する濃度プロフィールが測定および／または計算されて陽性／陰性検出および／または完全な分析物定量化を与えることができる。
【００３３】
本発明での使用に特に好ましいチャネルは、「マイクロチャネル」である。本明細書中で用語「マイクロチャネル」は、低レイノルズ数操作を可能にする寸法を有するチャネル、すなわち、流体の動力学が慣性力よりもむしろ粘性力により支配されるものについて使用される。粘性力に対する慣性力の比とも時にいわれるレイノルズ数は、以下により与えられる。
【００３４】
$Re = \rho d^2 / \eta\tau + \rho u d / \eta$
ここに、ｕは速度ベクトル、ρは流体の密度、ηは流体の粘度、ｄはチャネルの特性寸法、τは速度が変化する時間スケールである（ここでｕ／τ＝δｕ／ｄｔ）。用語「特性寸法」はここで、当業で周知の通り、レイノルズ数を決定する寸法について使用されている。円筒形チャネルの場合、それは直径である。矩形チャネルの場合、それは基本的に幅および深さのうちの小さい方に依存する。Ｖ字形チャネルの場合、それは「Ｖ」の頂部の幅に依存する、といったことになる。様々な形態学的チャネルに関するＲｅの計算は、流体力学の標準的な教科書に見ることができる（例えば、Ｇｒａｎｇｅｒ（１９９５）Ｆｌｕｉｄ Ｍｅｃｈａｎｉｃｓ，Ｄｏｖｅｒ，Ｎ．Ｙ．；Ｍｅｙｅｒ（１９８２）ｉｎｔｒｏｄｕｃｔｉｏｎ ｔｏ Ｍａｔｈｅｍａｔｉｃａｌ Ｆｌｕｉｄ Ｄｙｎａｍｉｃｓ，Ｄｏｖｅｒ，Ｎ．Ｙ．）。
【００３５】
定常状態（τ→∞）における流体の流れの挙動は、レイノルズ数Ｒｅ＝ρｕｄ／ηによって特徴づけられる。マイクロ加工された流体システムは、小サイズおよび低速度のために、たいてい低レイノルズ数レジーム（Ｒｅはほぼ１未満）にある。このレジームでは、乱流および二次流れ、従って流れ内部での混合を生じさせる慣性効果が無視でき、粘性効果が動力学を支配する。こうした条件下では、チャネル内の流れは一般に層状である。
【００３６】
レイノルズ数が、チャネル寸法だけでなく、流体密度、流体粘度、流体速度および、速度が変化する時間スケールにも依存するので、チャネル直径の絶対上限は明確には規定されない。実際、良好に設計されたチャネル幾何学形状によれば、乱流はＲ＜１００について、ことによるとＲ＜１０００について回避することができ、従って相対的に大きいチャネルサイズを有する高処理能システムが可能である。好ましいチャネル特性寸法範囲は、約０．５μｍないし１００ｍｍである。約１μｍ～約１００μｍの特性寸法のチャネルレンジが特に好ましく、約５μｍ～約１００μｍが最も好ましい。より好ましいレンジは、約５μｍないし５０μｍである。

【００３７】
本発明のデバイスは低レイノルズ数操作に制限される必要はない。結合プローブが広く離間され、それゆえ放出された分析物が流れにおいて広く離間される場合、異なる分析物が互いの信号を「オーバラップしたり」マスクすることなく、かなりの対流混合がチャネルにおいて生起し得る。さらに、２分析物のかなりの混合が生起し得て、２分析物のピーク濃度間に著しい（例えば、統計的に有意な）空間分離が存在する限り、信号は区別可能であり、各分析物の検出が行い得ることが理解されよう。しかし、分析物が混合し合うにつれて、各個別の分析物の定量化は次第により難しくなるかもしれない。それにもかかわらず、そうした状況でさえ、各分析物に積分信号の近似を与えるために濃度ピークの位置およびフォールオフ率に基づく分析物の空間分布を評価またはモデル化することによって、定量化を得ることができる。

【００３８】
上述の通り、上述の混合要求条件が満たされる限り、あらゆるチャネル構成が適格である。従って、適切なチャネルには、以下に限定されないが、対向する隆壁、無蓋溝、閉渠などによって形成されるチャネルを含む。チャネルは、例えば円形、方形、矩形、三角形、ｖ字状、ｕ字状、六角形、八角形、不規則など、事実上あらゆる断面を有することができる。本発明において使用されるチャネルは連続的である必要もない。従って例えば、チャネルは、多孔性粒子の集合体、共重合体または架橋重合体などによって形成することができる。

【００３９】
その中を通過する溶液に対し材料が本質的に安定している限り、あらゆるチャネル材料が本発明の実施に適する。好ましい材料は、結合相手と結合できるかまたは結合するようにに誘導体化することができる、または結合相手のリンカーである。さらに、好ましい実施形態において、材料は、それが分析物と実質的に結合しないように選択および／または改質される。また好ましい材料は、プローブを固定することが望まれる所以外の領域においてプローブと結合しないか、または別様に相互作用しない。

【００４０】
特に好ましい材料は、以下に限定されないが、ガラス、ケイ素、石英または他の鉱物、プラスチック、セラミックス、金属、紙、メタクロイド、半導体、セメントなどを含む。さらに、タンパク質（例えばゼラチン）、リボ多糖類、ケイ酸塩、アガロースおよびポリアクリルアミドといったゲルを形成する物質を使用することができる。天然および合成両方の、多種多様な有機重合体および無機重合体が、固体表面の材料として使用され得る。例示的な重合体は、ポリエチレン、ポリプロピレン、ポリ（４－メチルブテン）、ポリスチレン、ポリメタクリレート、ポリ（エチレンテレフタレート）、レーヨン、ナイロン、ポリ（ビニルブチレート）、ポリビニリデンジフルオリド（ＰＶＤＦ）、シリコン、ポリホルムアルデヒド、セルロース、酢酸セルロース、ニトロセルロースなどを含む。

【００４１】
導電性または半導電性基板の場合、好ましくは基板に絶縁層が存在する。これは、デバイスが電気的要素を組み込む場合（例えば、電気的流体方向システム、センサなど、または電気浸透力を使用してシステムの周りで材料を移動させる）、特に重要である。重合体基質の場合、基質材料は、それらが意図される用途に応じて、硬質、半硬質、または非硬質、不透明、半透明または透明とすることができる。例えば、光学的または視覚的検出要素を含むデバイスは一般に、その検出を可能にするか、または少なくとも助成するために、少なくとも部分的に、透明材料により製作される。あるいはまた、例えばガラスまたは石英の透明な窓が、こうした形式の検出要素についてデバイスに据り入れられ得る。付加的に、重合体材料は、直鎖または枝分かれ主鎖を有し、架橋されるか、または非架橋とすることができる。特に好ましい重合体材料の例は、例えばポリジメチルシロキサン（ＰＤＭＳ）、ポリウレタン、ポリ塩化ビニル（ＶＰＣ）、ポリスチレン、ポリスルホン、ポリカーボネートなどを含む。

【００４２】

チャネルはより大きい物体の構成要素とすることができる。従って、チャネルは、多数のチャネルを得るために１個以上の他のチャネルと組み立てることができ、それによって複数の異なるアッセイを同時に実行することができる。チャネルは、適切な液体取扱および／または検出および／またはサンプル取扱／適用機能を含む器械の構成要素とすることができる。

【００４３】

また、チャネルは、本発明のアッセイを実行する器械に適宜「プラグイン」できる再使用可能または使い捨てのユニットとして製作することができる。チャネルは、以下に限定されないが、マイクロ滴定皿（例えば、ＰＶＣ、ポリプロピレンまたはポリスチレン）、試験管（ガラスまたはプラスチック）、計量棒（例えばガラス、ＰＶＣ、ポリプロピレン、ポリスチレン、ラテックスなど）、マイクロ遠心分離管、またはガラス、シリカ、プラスチック、金属または重合体ビーズを含む、多種多様な物体のうちのいずれか１個以上に備え得ることが理解される。

【００４４】

特に好ましい実施形態では、１個以上のチャネルが、キャピラリー管（例えばキャピラリー電気泳動管）として、ガラスまたはケイ素スライドに、キャピラリーチャネルとして設けられるか、または液体の流れの制御、サンプルの適用および／または信号の検出のためのオンボード回路要素を有する「集積回路」の要素として製作される。最も好ましい実施形態において、チャネルは、本明細書中の実施例において例示したように、例えばキャピラリー電気泳動管などのキャピラリーとして備えられる。

【００４５】

（２）チャネル製作）

本発明のチャネルを製作する方法は、当業者には公知である。例えば、チャネルが１個以上のキャピラリーから形成される場合、キャピラリーは市販業者（例えばＰｏｌｙｍｉｃｒｏｎ Ｔｅｃｈｎｏｌｏｇｉｅｓ，Ｔｕｃｓｏｎ，Ａｚ）から購入するか、または従来のキャピラリー「引抜き」械によって引抜きまたは押出しすることができる。

【００４６】

表面上にチャネルを製作する場合、それらは標準の技法によって形成でき、例えば、機械加工、成形、彫刻、エッチング、積層、押出または堆積などが可能である。

【００４７】

好ましい１実施形態において、チャネルは、固体電子産業で公知のマイクロマシニングプロセス（例えばフォトリソグラフィ）を用いて製作される。通例、マイクロデバイス、例えばマイクロチャネルは、集積回路を製造するために使用される半導体ウェーハの形で広範に入手可能な結晶ケイ素といった半導体基板から、またはガラスから作成される。材料の共通性のため、半導体ウェーハ基板からのマイクロデバイスの製作は、集積回路（ＩＣ）製造のために半導体加工業によって開発された表面エッチング技法およびバルクエッチング技法両方の幅広い経験を活用できる。

【００４８】

ＩＣ製造において半導体ウェーハに薄い表面パターンを形成するために使用される表面エッチングは、可動要素を作成するために半導体材料の薄層の犠牲アンダーカットエッチングを可能にするように修正できる。ＩＣ製造において一般に、異方性エッチングプロセスを用いてウェーハに深いトレンチを形成する際に使用されるバルクエッチングは、マイクロデバイスにおいてエッジまたはトレンチを精密に機械加工するために使用することができる。ウェーハの表面エッチングおよびバルクエッチングは両方とも、ウェーハからマスクされていない材料を取り去るために水酸化カリウム溶液といった化学薬品を使用する、「ウェット処理」により行うことができる。マイクロデバイス作成には、各種チャネル要素を形成するために、材料の示差的な結晶方位に頼る、または電気化学的エッチストップの使用に依存する、異方性ウェット処理技法を使用することさえ可能である。

【００４９】

マイクロデバイス設計の相当の自由を許す別のエッチング処理技法は、一般に「ドライエ

ッチング処理」として知られている。この処理技法は、微細構造の異方性エッチングに特に適する。ドライエッチング処理は、ウェーハ原子を気相に移すためにウェーハを高エネルギー原子またはイオンで衝撃する高異方性スパッタリングプロセスから（例えば、イオンビームミリング）、揮発性反応生成物の形成を誘起するために化学的反応性イオンを含んでいるプラズマストリームをウェーハに対して誘導するやや等方性の低エネルギープラズマ技法にまで及ぶ、多くの気相またはプラズマ相エッチング技法を含む。

【００５０】

高エネルギースパッタリング技法と低エネルギープラズマ技法との中間には、反応性イオンエッチングとして知られる特に有用なドライエッチングプロセスがある。反応性イオンエッチングは、同時的なスパッタリングおよびプラズマエッチングのためにイオン含有プラズマストリームを半導体または他のウェーハに対して誘導することを伴う。反応性イオンエッチングは、ウェーハとの反応性プラズマイオンの接触に応答した気相反応生成物の形成のために反応性プラズマイオンを供しながらも、スパッタリングに関係する異方性の利益のいくつかを保持する。実際、ウェーハ材料除去の速度は、単独で行うスパッタリング技法または低エネルギープラズマ技法のどちらか一方に対して著しく増強される。従って、反応性イオンエッチングは、相対的に高い異方性エッチングレートが持続可能であることにより、マイクロデバイス作成のための優れたエッチングプロセスとなる可能性を有する。上述のマイクロマシニング技法は、他の多くのことと同様、当業者には公知である（例えば、Ｃｈｏｕｄｈｕｒｙ（１９９７）Ｔｈｅ Ｈａｎｄｂｏｏｋ ｏｆ Ｍｉｃｒｏｌｉｔｈｏｇｒａｐｈｙ，Ｍｉｃｒｏｍａｃｈｉｎｉｎｇ，ａｎｄ Ｍｉｃｒｏｆａｂｒｉｃａｔｉｏｎ，Ｓｏｃ．Ｐｈｏｔｏ－Ｏｐｔｉｃａｌ Ｉｎｓｔｒｕ．Ｅｎｇｉｎｅｅｒ，Ｂａｒｄ ＆ Ｆａｕｌｋｎｅｒ（１９９７）Ｆｕｎｄａｍｅｎｔａｌｓ ｏｆ Ｍｉｃｒｏｆａｂｒｉｃａｔｉｏｎ参照）。さらに、ケイ素またはホウケイ酸ガラスチップでのマイクロマシニング技法の使用の実例は、米国特許第５，１９４，１３３号、同第５，１３２，０１２号、同第４，９０８，１１２号および同第４，８９１，１２０号に見ることができる。

【００５１】

１実施形態において、チャネルは、ケイ素（１００）ウェーハにおいて、チャネルおよび接続をパターン形成するために標準のフォトリソグラフィ技法を用いて微細加工される。エチレンジアミン、ピロカテコール（ＥＤＰ）が２段エッチングに使用され、閉液系を付与するためにパイレックス（登録商標）（Ｐｙｒｅｘ）７７４０カバープレートをケイ素の面に陽極接合することができる。この場合、液体接続はケイ素の背部に作ることができる。

【００５２】

上述の通り、好ましい実施形態において、チャネルは、ガラス、石英または、キャピラリー電気泳動管といった他のキャピラリーから製作することができる。

【００５３】

他の実施形態では、チャネルは、チャネル壁を形成するために基板に材料を堆積させることによって（例えば、スパッタリングまたは他の溶着技術を用いて）製作できるか、またはチャネルは材料において注型／成形され得る。注型／成形チャネルは、以下に限らないが、様々な金属、プラスチックまたはガラスを含む、多種多様な材料から容易に製作される。特定の好ましい実施形態において、チャネルは各種エラストマー（例えば、アルキル化クロロスルホン化ポリエチレン（Ａｃｓｉｕｍ（登録商標））、ポリオレフィンエラストマー（例えばＥｎｇａｇｅ（登録商標））、クロロスルホン化ポリエチレン（例えばＨｙｐａｌｏｎ（登録商標））、ペルフルオロエラストマー（例えばＫａｒｒｅｚ（登録商標））、ネオプレンポリクロロプレン、エチレンープロピレンージエンターポリマー（ＥＰＤＭ）、塩素化ポリエチレン（例えばＴｙｒｉｎ（登録商標））、各種シロキサン重合体（例えばポリジメチルシロキサンなど）で注型される。

【００５４】

（Ｂ）結合相手

好ましい実施形態において、本発明で使用されるチャネルは、１以上の表面に固定された１個以上の生物学的「結合相手」を保持する。生物学的「結合相手」または「結合ペア」の構成員は、抗体－抗原、レクチン－炭水化物、核酸－核酸、ビオチン－アビジンなどといった結合複合体を形成するために、他の分子と特異的に結合する分子または組成をいう。

【００５５】

用語「特異的に結合する」は、本明細書中で使用されるように、生体分子（例えば、タンパク質、核酸、抗体など）を指す時に、タンパク質および他の生物学的製剤の生体分子異種集団の存在を決定づける結合反応をいう。従って、指定された条件下で（例えば、抗体の場合におけるイムノアッセイ条件、または核酸の場合におけるストリンジェントハイブリダイゼーション条件）、指定のリガンドまたは抗体は、その特定の「標的」（例えば、タンパク質または核酸）と結合し、他の分子と有意な量で結合しない。

【００５６】

本発明において使用される結合相手は、識別／定量化される標的に基づいて選択される。従って、例えば、標的が核酸である場合、結合相手は、好ましくは核酸または核酸結合タンパク質である。標的がタンパク質である場合、結合相手は好ましくは、そのタンパク質と特異的に結合するレセプター、リガンドまたは抗体である。標的が糖類または糖タンパク質である場合、結合相手は、好ましくはレクチンなどである。

【００５７】

適格な結合相手（捕獲剤）は、以下に限らないが、核酸、タンパク質、レセプター結合タンパク質、核酸結合タンパク質、レクチン、糖類、糖タンパク質、抗体、脂質などを含む。そのような結合相手の合成または単離方法は、当業者には公知である。

【００５８】

（１）結合相手（捕獲剤）の調製

（ａ）核酸）

本発明において結合相手として使用するための核酸は、当業者に公知の多数の方法のいずれかに従って製造または単離することができる。１実施形態では、核酸は、単離された自然発生核酸（例えば、ゲノムＤＮＡ、ｃＤＮＡ、ｍＲＮＡなど）とすることができる。自然発生核酸を単離する方法は当業者には公知である（例えば、Ｓａｍｂｒｏｏｋら（１９８９）Ｍｏｌｅｃｕｌａｒ Ｃｌｏｎｉｎｇ－Ａ Ｌａｂｏｒａｔｏｒｙ Ｍａｎｕａｌ（第２版），１－３巻，Ｃｏｌｄ Ｓｐｒｉｎｇ Ｈａｒｂｏｒ Ｌａｂｏｒａｔｏｒｙ，Ｃｏｌｄ Ｓｐｒｉｎｇ Ｈａｒｂｏｒ，Ｎ．Ｙ．参照）。

【００５９】

しかし、好ましい実施形態において、核酸は例えば化学合成によって新規に（ｄｅ ｎｏｖｏ）作成される。好ましい実施形態では、核酸（例えばオリゴヌクレオチド）は、Ｎｅｅｄｈａｍ－ＶａｎＤｅｖａｎｔｅｒら（Ｎｅｅｄｈａｍ－ＶａｎＤｅｖａｎｔｅｒら（１９８４）Ｎｕｃｌｅｉｃ Ａｃｉｄｓ Ｒｅｓ．，１２：６１５９－６１６８）に記載の通り自動合成デバイスを用いるなどして、ＢｅａｕｃａｇｅおよびＣａｒｕｔｈｅｒｓ（Ｂｅａｕｃａｇｅ ａｎｄ Ｃａｒｕｔｈｅｒｓ（１９８１），Ｔｅｔｒａｈｅｄｒｏｎ Ｌｅｔｔｓ．，２２（２０）：１８５９－１８６２）が記載した、固相ホスホラミダイトトリエステル法に従って化学的に合成される。必要な場合、オリゴヌクレオチドの精製は一般に、ＰｅａｒｓｏｎおよびＲｅｇｎｉｅｒ（Ｐｅａｒｓｏｎ ａｎｄ Ｒｅｇｎｉｅｒ（１９８３）Ｊ．Ｃｈｒｏｍ．２５５：１３７－１４９）に記載の通り、ネイティブアクリルアミドゲル電気泳動またはアニオン交換ＨＰＬＣのどちらか一方によって実行される。合成オリゴヌクレオチドの配列は、ＭａｘａｍおよびＧｉｌｂｅｒｔ（Ｍａｘａｍ ａｎｄ Ｇｉｌｂｅｒｔ（１９８０）ｉｎ Ｇｒｏｓｓｍａｎ ａｎｄ Ｍｏｌｄａｖｅ（編）Ａｃａｄｅｍｉｃ Ｐｒｅｓｓ，Ｎｅｗ Ｙｏｒｋ，Ｍｅｔｈ．Ｅｎｚｙｍｏｌ．６５：４９９－５６０）の化学的減成法を用いて確認できる。

【００６０】

（ｂ）抗体／抗体フラグメント）

結合相手（捕獲剤）として使用するための抗体または抗体フラグメントは、当業者に公知の多くの方法によって製造することができる（例えば、Harlow & Lane（1988）Antibodies：A Laboratory Manual，Cold Spring Harbor Laboratory，and Asai（1993）Methods in Cell Biology 第37巻：Antibodies in Cell Biology，Academic Press，Inc．N．Y．参照）。1方法において、抗体は、認識／捕獲したいと望むエピトープを含んでいる免疫原により動物（例えばウサギ）を免疫にすることによって産生される。多数の免疫原が特異反応性抗体を産生するために使用できる。組換えタンパク質は、モノクロナール抗体またはポリクローナル抗体の産生に好ましい免疫原である。また天然に存在するタンパク質も、純粋または不純な形態のどちらか一方で使用できる。合成ペプチドも同様に、標準のペプチド合成化学によって作成される（例えば、BaranyおよびMerrifield，Solid-Phase Peptide Synthesis；3-284頁，The Peptides：Analysis，Synthesis，Biology，第2巻：Special Methods in Peptide Synthesis，Part A．，Merrifieldら（1963）J．Am．Chem．Soc．，85：2149-2156，and Stewartら（1984）Solid Phase Peptide Synthesis，第2版、Pierce Chem．Co．，Rockford，Ill参照）。

【0061】

ポリクローナル抗体の産生方法は当業者に周知である。簡単にいえば、好ましくは精製した細胞膜構成分である免疫原を、アジュバントと混合し、動物を免疫にする。その動物の免疫原製剤に対する免疫反応を、試験ブリードを行い、細胞骨格成分および試験組成への反応性の力価を決定することによって監視する。免疫原に対する抗体の適切に高い力価が得られた場合、血液を動物から採集し、抗血清が準備される。所要の場合、細胞骨格成分に反応性の抗体について濃縮するために抗血清のさらなる画分を行うことができる。（前掲Harlow & Laneを参照）。

【0062】

モノクロナール抗体は、当業者にはなじみの各種技法によって得ることができる。簡単にいえば、所望の抗原で免疫にされた動物からの脾臓細胞を、一般に骨髄腫細胞との融合によって、不死化する（KohlerおよびMilstein（1976）Eur．J．Immunol．6：511-519参照）。不死化の代替方法は、エプスタイン・バーウイルス、癌遺伝子もしくはレトロウイルスまたは当業で周知の他の方法による形質転換を含む。単一の不死化細胞から生じるコロニーを、抗原について所望の特異性およびアフィニティーの抗体の産生のためにスクリーニングし、そしてそのような細胞により産生されるモノクロナール抗体の収率は、脊椎動物宿主の腹膜腔への注入を含む各種技法によって増強できる。あるいはまた、Husら（1989）Science，246：1275-1281）により概説された一般プロトコルに従ってヒトB細胞からDNAライブラリーをスクリーニングすることによって、モノクロナール抗体またはその結合フラグメントをコードするDNA塩基配列を単離することも可能である。

【0063】

例えば一本鎖抗体（scFvまたはその他）などの抗体フラグメントも、ファージディスプレイ技術を用いて産生／選択することができる。バクテリアを感染させるウイルス（バクテリオファージまたはファージ）の表面に抗体フラグメントを発現できる能力は、10^{9}を超える非結合クローンのライブラリーから単一の結合抗体フラグメントを単離することを可能にする。ファージの表面に抗体フラグメントを発現させる（ファージディスプレイ）ために、抗体フラグメント遺伝子がファージ表面タンパク質（pIII）をコードする遺伝子に挿入され、そして抗体フラグメント-pIII融合タンパク質がファージ表面にディスプレイされる（McCaffertyら（1990）Nature，348：552-554；Hoogenboomら（1991）Nucleic Acids R

es，19：4133－4137）。

【0064】

ファージの表面上の抗体フラグメントが機能性であるので、抗原結合抗体フラグメントを保持するファージは、抗原アフィニティークロマトグラフィー（McCaffertyら（1990）Nature，348：552－554）によって非結合ファージから単離できる。抗体フラグメントのアフィニティーに依存して、一回のアフィニティー選別について20倍～1，000，000倍の濃縮率が得られる。しかし、溶離されたファージにバクテリアを感染させることによって、より多くのファージを増殖させ、そしてもう1回の選別を受けさせることができる。このようにして、1回での1000倍の濃縮が2回の選別において1，000，000倍になり得る（McCaffertyら（1990）Nature，348：552－554）。従って濃縮が低い時でさえ（Marksら（1991）J．Mol．Biol．222：581－597）、複数回のアフィニティー選別は、稀少ファージの単離をもたらし得る。抗原のファージ抗体ライブラリーの選別が濃縮を結果として生じるので、大多数のクローンは、3～4回程度の選別の後に抗原を結合する。従って、比較的少数（数百）のクローンを抗原との結合のみについて分析する必要がある。

【0065】

ヒト抗体は、ファージ上で非常に大きくかつ多様なV遺伝子レパートリーをディスプレイすることによって従来の免疫法によらず産生できる（Marksら（1991）J．Mol．Biol．222：581－597）。1実施形態において、ヒトの末梢血リンパ球に存在する自然のV$_H$およびV$_L$レパートリーが、非免疫化ドナーからPCRによって単離された。V遺伝子レパートリーは、PCRを用いて無作為に共にスプライスされ、scFv遺伝子レパートリーを作製し、これはファージベクターにクローンされて3000万ファージ抗体のライブラリーを作製した（同書）。この単一の「未処理の」ファージ抗体ライブラリーから、結合抗体フラグメントが、ハプテン、多糖類およびタンパク質を含む、17を超える異なる抗原に対して単離された（Marksら（1991）J．Mol．Biol．222：581－597；Marksら（1993）．Bio／Technology．10：779－783：Griffithsら（1993）EMBO J．12：725－734；Clacksonら（1991）Nature．352：624－628）。抗体は、ヒトのサイログロブリン、免疫グロブリン、脂質壊死因子およびCEAを含む、自己タンパク質に対して産生された（Griffithsら（1993）EMBO J．12：725－734）。また、そのままの細胞で直接選別することによって細胞表面抗原に対する抗体を単離することも可能である。抗体フラグメントは、選別に使用される抗原について非常に特異的であり、1：M～100nMの範囲のアフィニティーを有する（Marksら（1991）J．Mol．Biol．222：581－597：Griffithsら（1993）EMBO J．12：725－734）。より大きなファージ抗体ライブラリーは、より大きい割合の抗原に対する高い結合アフィニティーの、より多数の抗体の単離を結果として生じる。

【0066】

（c）結合タンパク質）

1実施形態において、結合相手（捕獲剤）は結合タンパク質であり得る。適格な結合タンパク質は、以下に限らないが、レセプター（例えば、細胞表面レセプター）、レセプターリガンド、サイトカイン、転写因子および他の核酸結合タンパク質、成長因子などを含む。

【0067】

タンパク質は、天然源から単離するか、単離されたタンパク質から突然変異を誘発させるか、または新規に合成することができる。天然に存在するタンパク質を単離する手段は、当業者に公知である。そのような方法は、以下に限らないが、硫酸アンモニウム沈殿、アフィニティーカラム、カラムクロマトグラフィー、ゲル電気泳動などを含む、周知のタンパク質精製方法を含む（一般的には、R．Scopes，（1982）Protein

Purification, Springer-Verlag, N. Y.：Deutscher (1990) Methods in Enzymology第182巻：Guide to Protein Purification, Academic Press, Inc. N. Y. 参照)。

【0068】

タンパク質が標的を可逆的に結合する場合、振的を保持するアフィニティーカラムが、タンパク質をアフィニティー精製するために使用され得る。あるいは、タンパク質は、HISタグと組換え的に発現され、そしてNi2+／NTAクロマトグラフィーを用いて精製されることもできる。

【0069】

別の実施形態では、タンパク質は、標準の化学的ペプチド合成技法を用いて化学的に合成され得る。所望の配列が比較的短い場合、分子は、単一の連続的なポリペプチドとして合成され得る。より大きい分子が所望される場合、部分配列は（1以上の単位で）別個に合成され、その後、一方の分子のアミノ末端と他方の分子のカルボキシル末端との縮合によりペプチド結合を形成することによって融合できる。これは代表的に、商業用ペプチド合成機において単一のアミノ酸を結合するために使用されるのと同じ化学（例えば、Fmoc、Tboc）を用いて行われる。

【0070】

配列のC末端アミノ酸が不活性担体に固定された後、配列の残りのアミノ酸の順次的付加を伴う固相合成は、本発明のポリペプチドの化学合成のために好ましい方法である。固相合成の技法については、BaranyおよびMerrifield (Baranyおよび Merrifield (1962) Solid-Phase Peptide Synthesis：3-284頁, The Peptides：Analysis, Synthesis, Biology. 第2巻：Special Methods in Peptide Synthesis, Part A.)、Merrifieldら (Merrifieldら (1963) J. Am. Chem. Soc., 85：2149-2156)および、Stewartら (Stewartら (1984) Solid Phase Peptide Synthesis, 第2版, Pierce Chem. Co., Rockford, Ill) により記載されている。

【0071】

好ましい実施形態において、それは、組換えDNA方法論を用いて合成され得る。一般に、これは、結合タンパク質をコードするDNA塩基配列を作成する工程、特定のプロモーターの制御下の発現カセットにDNAを置く工程、宿主においてタンパク質を発現させる工程、発現したタンパク質を単離する工程、さらに必要な場合、タンパク質を再生させる工程を包含する。

【0072】

本発明の結合タンパク質または配列をコードするDNAは、例えば、適切な配列のクローニングおよび制限または、Narangらのホスホトリエステル法 (Narangら (1979) Meth. Enzymol. 68：90-99)、Brownらのホスホジエステル法 (Brownら (1979) Meth. Enzymol. 68：109-151)、Beaucageらのジエチルホスホラミダイト法 (Beaucageら (1981) Tetra. Lett., 22：1859-1862)および、米国特許第4,458,066号の固体担体法といった方法による直接的化学合成を含む、上述のような任意の適切な方法によって調製することができる。

【0073】

所望の結合タンパク質をコードする核酸配列は、大腸菌、他の細菌宿主、酵母、ならびにCOS、CHOおよびHeLa細胞株および骨髄腫細胞系などの様々な高次の真核細胞を含む、多様な宿主細胞において発現され得る。組換えタンパク質遺伝子は、個々の宿主について適切な発現調節配列と作動可能に連結され得る。大腸菌の場合、これは、T7、trpまたはλプロモーターといったプロモーター、リボソーム結合部位および、好ましく

は転写終結シグナルを含む。真核細胞の場合、調節配列はプロモーターおよび好ましくは、免疫グロブリン遺伝子、ＳＶ４０、サイトメガロウイルスなどから誘導されたエンハンサー、およびポリアデニレーション配列を含み、また、スプライスドナーおよびアセプター配列を含むこともある。

【００７４】

プラスミドは、大腸菌の場合の塩化カルシウム形質転換および、哺乳動物細胞の場合のリン酸カルシウム処理または電気穿孔といった公知の方法によって、選択された宿主細胞に移入され得る。プラスミドにより形質転換される細胞は、ａｍｐ遺伝子、ｇｐｔ遺伝子、ｎｅｏ遺伝子およびｈｙｇ遺伝子といったプラスミドに含まれる遺伝子により与えられる抗体への耐性によって選択されることができる。

【００７５】

一旦発現されると、組換え結合タンパク質は、上述のような当業の標準のプロトコルに従って精製され得る。

【００７６】

（ｄ）糖類および炭水化物）

他の結合相手としては糖類および炭水化物を含む。糖類および炭水化物は、天然源から単離するか、酵素により合成するかまたは、化学的に合成することができる。特定の少糖類構造の産生のための経路は、ｉｎ ｖｉｖｏでそれらを作製する酵素（グリコシルトランスフェラーゼ）の使用によるものである。そうした酵素は、少糖類のｉｎ ｖｉｔｒｏ合成のためのレジオ選択的およびステレオ選択的触媒として使用され得る（Ｉｃｈｉｋａｗａら（１９９２）Ａｎａｌ．Ｂｉｏｃｈｅｍ．２０２：２１５－２３８）。シアリルトランスフェラーゼは補助的なグリコシルトランスフェラーゼとの組合せで使用され得る。例えば、シアリルトランスフェラーゼとガラクトシルトランスフェラーゼとの組合せを使用することができる。所望の少糖類構造を合成するためにグリコシルトランスフェラーゼを使用する多くの方法が公知である。例示的な方法は、例えば、ＷＯ９６／３２４９１、Ｉｔｏら（Ｉｔｏら（１９９３）Ｐｕｒｅ Ａｐｐｌ．Ｃｈｅｍ．６５：７５３）ならびに、米国特許第５，３５２，６７０号、第５，３７４，５４１号および第５，５４５，５５３号に記載されている。酵素および基質は初期の反応混合物において組合せられるか、または代わりに、酵素および第２のグリコシルトランスフェラーゼ回路の試薬を、最初のグリコシルトランスフェラーゼ回路が完成に近づいた時に反応媒体に添加することができる。単一の容器において２つのグリコシルトランスフェラーゼ回路を順番に実施することによって、全体の収率は、中間種が単離されるプロトコルよりも改善される。

【００７７】

化学合成の方法はＺｈａｎｇ（Ｚｈａｎｇら（１９９９）Ｊ．Ａｍ．Ｃｈｅｍ．Ｓｏｃ．，１２１（４）：７３４－７５３）により記載されている。簡単にいえば、この方法では、糖ベースの基本単位のセットは、異なる保護基をプレロードされた各基本単位により作成される。基本単位は各保護基の反応性によって格付けされる。その後コンピュータプログラムが、最速のものから最緩のものまで一連の反応が所望の化合物を産生するように、どの基本成分が反応に追加されなければならないかを正確に決定する。

【００７８】

（２）チャネルへの結合相手の付着）

生体分子を多様な固体表面に固定化する多くの方法が当該分野で公知である。所望の成分は、共有結合されるかまたは、特異的または非特異的な結合を介して非共有結合で固定され得る。

【００７９】

化合物と表面との間に共有結合が望まれる場合、表面は通常、多官能性であるか、または多官能化できるものである。表面に存在しそして結合に使用され得る官能基は、カルボン酸、アルデヒド、アミノ基、シアノ基、エチレン基、ヒドロキシル基、メルカプト基などを含み得る。多様な化合物を合種表面に連結する方法は、周知であり、そして文献に豊富に例示されている。例えば、Ｉｃｈｉｒｏ Ｃｈｉｂａｔａ（Ｉｃｈｉｒｏ Ｃｈｉｂａ

ta（1978）Immobilized Enzymes, Halsted Press, New York）およびCuatrecasas（Cuatrecasas, （1970）J. Biol. Chem. 245：3059）を参照されたい。

【0080】

共有結合に加え、アッセイ成分を非共有結合で結合する各種方法を使用することができる。非共有結合は一般に、表面への化合物の非特異的な吸着である。通常、表面は、標識されたアッセイ成分の非特異的な結合を防止するために第2の化合物でブロックされる。あるいは、表面を、それが1成分と非特異的に結合するが別のものとはほとんど結合しないように設計する。例えば、コンカナバリンAといったレクチンを保有する表面は、炭水化物含有化合物とは結合するが、グリコシル化を欠く標識されたタンパク質とは結合しないであろう。アッセイ成分の非共有結合での固定に使用するための各種固体表面は、米国特許第4，447，576号および同第4，254，082号において概説されている。

【0081】

結合相手が核酸またはポリペプチドである場合、分子は、in situで化学的に合成することができる。これは、光不安定性保護基を通常の保護基（例えば、核酸合成において使用されるジメトキシトリチル基（DMT））で置換する実質的に標準の化学合成方法を含む。離散した位置でのマイクロチャネルの照射は、モノマー（例えば、アミノ酸またはヌクレオチド）と、照射された部位の成長ポリペプチドまたは核酸との選択的結合を結果として生じる。光指向性重合体合成の方法は当業者には周知である（例えば、米国特許第5，143，854号、PCT公開番号WO90／15070、WO92／10092およびWO93／09668、さらにFodorら（1991）Science, 251, 767－77参照）。

【0082】

好ましい実施形態において、結合相手はリンカー（例えば、ホモ二官能性またはヘテロ二官能性リンカー）を使用して固定され得る。生物学的結合相手を結合するために適格なリンカーは、当業者に周知である。例えば、タンパク質または核酸分子は、以下に限らないが、ペプチドリンカー、直鎖または枝分れ鎖炭素鎖リンカーを含む多様なリンカーのうちのいずれかによって、または複素環式炭素リンカーによって結合され得る。N－エチルマレイミドの活性エステルといったヘテロ二官能性架橋試薬は、広く使用されている（例えば、Lernerら（1981）Proc. Nat. Acad. Sci. USA, 78：3403－3407）、Kitagawaら（Kitagawaら（1976）J. Biochem., 79：233－236）、BirchおよびLennox（Birch and Lennox（1995）第4章, Monoclonal Antibodies：Principles and Applications, Wiley－Liss, N. Y.）参照）。

【0083】

好ましい1実施形態では、結合相手は、ビオチン／アビジン相互作用を用いて固定される。この実施形態において、光不安定性保護基を有するビオチンまたはアビジンが、チャネルに配置され得る。個別の位置のチャネルの照射は、その位置におけるチャネルへのビオチンまたはアビジンの結合を生じる。その後、各々のビオチンまたはアビジンを保持する結合剤がチャネルに配置されて、それは各々の結合相手と結合し、照射部位において局在化される。このプロセスが、結合相手を固定することが望まれる各個別位置において繰り返され得る。

【0084】

別の適切な光化学的結合方法は、Sigristら（Sigristら（1992）Bio／Technology, 10：1026－1028）が記載している。この方法では、有機表面または無機表面とのリガンドの相互作用は、リンカー分子として働くトリフルオロメチルーアリールージアジリンを生成するカルベンを有する光活性化可能な重合体によって伸介される。350nmにおけるアリールージアジリン官能基の光活性化は高反応性カルベンを産出し、そして共有結合はリガンドおよび不活性表面両方への同時カルベン

挿入によって達成される。従って、反応性官能基はリガンドまたは支持材料のどちらにおいても必要ではない。

【００８５】

最も好ましい実施形態において、溶融シリカ（ｆｕｓｅｄ　ｓｉｌｉｃａ）キャピラリー（内径５０μｍ）は、有機塗料で溶融シリカ表面を被覆するためにエポキシ樹脂（Ｅｐｏｔｅｋ３５０）の溶液を塗被される。表面の有機塗料は、キャピラリー壁へのＤＮＡ吸着を最小限にするだけではなく、ＤＮＡプローブが直接固定化できる疎合化表面を提供する。エポキシ樹脂でキャピラリー表面を被覆するプロトコルは、Ｌｉｕら（Ｌｉｕら（１９９６）Ｊ．Ｃｈｒｏｍａｔｏｇｒ．７２３：１５７－１６７）が記載している。簡単にいえば、キャピラリーを、最初にアセトンで１５分間すすぎ洗いした後、２０ｐｓｉの窒素圧下で１時間、１００℃でオーブンにて乾燥した。エポキシ樹脂３１４ＮＤ（Ｅｐｏ－Ｔｅｋ，Ｂｉｌｌｅｒｉｃａ，ＭＡ）を、エポキシ樹脂混合物のアセトン溶液を吸引することによってキャピラリー表面に動的に被覆した。残留溶剤は、室温で３０分間、窒素を流すことによってエポキシ樹脂被覆キャピラリーから除去された。エポキシ樹脂塗料を８０℃で３０分間、その後２０ｐｓｉの窒素圧下で１５０℃で２時間、架橋した。塗被したキャピラリーは、使用前に３０分間緩衝液で洗浄する。

【００８６】

その後、エポキシ樹脂被覆キャピラリーの１ｃｍセクションに、特定のＤＮＡプローブ溶液を流す。ＤＮＡプローブ溶液は、ＤＮＡプローブを疎水性相互作用および静電相互作用によってキャピラリー壁と結合させるために一晩キャピラリー片と反応させる。他のＤＮＡプローブは、同様の１ｃｍ長の被覆キャピラリー片に同様にして固定される。一旦それがキャピラリー壁に固定化されると、それらのハイブリダイゼーション領域は、脱イオン水ですすいだ後、異なる位置に異なる結合相手を有するキャピラリーバイオセンサに組み立てる準備が整う。

【００８７】

（Ｃ）分析物検出方法）

生物学的分子検出の事実上あらゆる方法が、本発明の方法に従って使用されることができる。各種分析物の正体が、チャネル内を動いている流れにおけるそれらの空間位置によって決定されるので、個々の分析物に関して異なる標識化システムの必要がない。それどころか、このアッセイシステムの１つの利点は、分析物に標識を付ける必要がまったくないということである。

【００８８】

分析物を検出する方法は当業者に周知である。分析物が（例えば、放射性、蛍光性、磁気または質量標識により）標識化されている場合、分析物は、標識を検出することによって検出される。しかし、好ましい実施形態では、分析物は標識化されず、そして好ましい検出方法は分析物に付けられた標識の使用に依存しない。そうした検出方法は、以下に限らないが、光信号の検出（例えば、放射および／または吸収分光学）、電気および磁気的信号の検出、分析物を含有する媒体の電気的特性（例えば、コンダクタンス／抵抗、キャパシタンス、インピーダンスなど）の変化の検出を含む。

【００８９】

単純な１実施形態では、分析物を含有する流体の光学的吸収が、（例えば、標準の紫外線）検出器により監視される。しかし、好ましい実施形態では、電気分析的検出器が使用される。最も好ましい実施形態では、電気分析的検出器は（例えばシヌソイド）ボルタンメトリーを使用する。

【００９０】

特に好ましい実施形態において、シヌソイドボルタンメトリーは、少量の目的の分析物をボルタンメトリー電極に供給することを含む。正弦（または他の経時変化する）電圧が電極に適用される。経時変化する（例えば正弦）電圧は、所定の周波数の単一周期で目的のレドックス種の形式電位を掃引するために十分に大きい振幅を有する。正弦波電圧に対する分析物の応答は、経時変化電圧の基本周波数の高調波で選択的に検出される。経時変化

ボルタンメトリーを実行する方法は、米国特許第５，６５０，０６１号およびその中で引用された参考文献において提供されている。

【００９１】

特に好ましい実施形態はシヌソイドボルタンメトリーを利用するが、他のボルタンメトリー方法も本発明に良好に適する。上述の通り、経時変化ボルタンメトリー方法が特に好ましく、そしてそうしたボルタンメトリー方法は正弦波の経時変化する電圧の使用に制限されない。また他の波形も適切である。そのような方法は、以下に限らないが、方形波および三角波（ｔｒｉａｎｇｌｅ　ｗａｖｅ）の使用を含む。そうした経時変化ボルタンメトリー方法は当業者に周知である（例えば、Ｃｕｌｌｉｓｏｎ　ａｎｄ　Ｋｕｈｒ　（１９９６）　Ｅｌｅｃｔｒｏａｎａｌｙｓｉｓ，　７（１）：　１－６参照）。

【００９２】

本発明の発見は、シヌソイドボルタンメトリー検出と空間的にコードされた分析物単離との組合せが、複雑なサンプル（例えば血清）において極めて低いレベルで高度に特異的な分析物の検出／定量を提供することであった。

【００９３】

（ＩＩＩ．統合アッセイデバイス）

薬品製造、環境分析、医学診断および基礎実験室分析において使用される最新の化学分析システムは、好ましくは全自動化の能力がある。そうした総合分析システム（ＴＡＳ）（Ｆｉｌｉｐｉｎｉら（１９９１）Ｊ．Ｂｉｏｔｅｃｈｎｏｌ．１８：１５３；Ｇａｒｎら（１９８９）Ｂｉｏｔｅｃｈｎｏｌ．Ｂｉｏｅｎｇ．３４：４２３；Ｔｓｈｕｌｅｎａ（１９８８）Ｐｈｙｓ．Ｓｃｒ．Ｔ２３：２９３；Ｅｄｍｏｎｄｓ（１９８５）Ｔｒｅｎｄｓ　Ａｎａｌ．Ｃｈｅｍ．４：２２０；Ｓｔｉｎｓｈｏｆｆら（１９８５）Ａｎａｌ．Ｃｈｅｍ．５７：１１４Ｒ；Ｇｕｉｂａｕｌｔ（１９８３）Ａｎａｌ．Ｃｈｅｍ　Ｓｙｍｐ．Ｓｅｒ．１７：６３７；Ｗｉｄｍｅｒ（１９８３）Ｔｒｅｎｄｓ　Ａｎａｌ．Ｃｈｅｍ．２：８）は、システムへのサンプルの導入から、システム内のサンプルの輸送、サンプル調製、単離、精製および、データ収集および評価を含む検出に及ぶ機能を自動的に実行する。

【００９４】

近年、サンプル調製技術は、小型化された形態に首尾よく縮小されている。従って例えば、ガスクロマトグラフィー（Ｗｉｄｍｅｒら（１９８４）Ｉｎｔ．Ｊ．Ｅｎｖｉｒｏｎ．Ａｎａｌ．Ｃｈｅｍ．１８：１）、高圧液体クロマトグラフィー（Ｍｕｌｌｅｒら（１９９１）Ｊ．Ｈｉｇｈ　Ｒｅｓｏｌｕｔ．Ｃｈｒｏｍａｔｏｇｒ．１４：１７４；Ｍａｎｚら（１９９０）Ｓｅｎｓｏｒｓ　＆　Ａｃｔｕａｔｏｒｓ　Ｂ１：２４９；Ｎｏｖｏｔｎｙら編（１９８５）Ｍｉｃｒｏｃｏｌｕｍｎ　Ｓｅｐａｒａｔｉｏｎｓ：Ｃｏｌｕｍｎｓ，Ｉｎｓｔｒｕｍｅｎｔａｔｉｏｎ　ａｎｄ　Ａｎｃｉｌｌａｒｙ　Ｔｅｃｈｎｉｑｕｅｓ　Ｊ．Ｃｈｒｏｍａｔｏｇｒ．Ｌｉｂｒａｒｙ，第３０巻；Ｋｕｃｅｒａ編（１９８４）Ｍｉｃｒｏ－Ｃｏｌｕｍｎ　Ｈｉｇｈ　Ｐｅｒｆｏｒｍａｎｃｅ　Ｌｉｑｕｉｄ　Ｃｈｒｏｍａｔｏｇｒａｐｈｙ，Ｅｌｓｅｖｉｅｒ，Ａｍｓｔｅｒｄａｍ；Ｓｃｏｔｔ編（１９８４）Ｓｍａｌｌ　Ｂｏｒｅ　Ｌｉｑｕｉｄ　Ｃｈｒｏｍａｔｏｇｒａｐｈｙ　Ｃｏｌｕｍｎｓ：Ｔｈｅｉｒ　Ｐｒｏｐｅｒｔｉｅｓ　ａｎｄ　Ｕｓｅｓ，Ｗｉｌｅｙ，Ｎ．Ｙ．；Ｊｏｒｇｅｎｓｏｎらら（１９８３）Ｊ．Ｃｈｒｏｍａｔｏｇｒ．２５５：３３５：Ｋｎｏｘら（１９７９）Ｊ．Ｃｈｒｏｍａｔｏｇｒ．１８６：４０５；Ｔｓｕｄａら（１９７８）Ａｎａｌ．Ｃｈｅｍ．５０：６３２）および、キャピラリー電気泳動法（Ｍａｎｚら（１９９２）Ｊ．Ｃｈｒｏｍａｔｏｇｒ．５９３：２５３；Ｏｌｅｆｉｒｏｗｉｃｚら（１９９０）Ａｎａｌ．Ｃｈｅｍ．６２：１８７２；Ｓｅｃｏｎｄ　Ｉｎｔ’ｌ　Ｓｙｍｐ．Ｈｉｇｈ－Ｐｅｒｆ．Ｃａｐｉｌｌａｒｙ　Ｅｌｅｃｔｒｏｐｈｏｒｅｓｉｓ（１９９０）Ｊ．Ｃｈｒｏｍａｔｏｇｒ．５１６；Ｇｈｏｗｓｉら（１９９０）Ａｎａｌ．Ｃｈｅｍ．６２：２７１４）は、小型化された形態に首尾よく縮小されている。

【００９５】

同様に、別の実施形態において、本発明は、多数の分析物を検出および／または定量す

る統合アッセイデバイス（例えばＴＡＳ）を提供する。アッセイデバイスは、上述の通り固定された結合相手を備えるチャネルを含む。さらに、好ましい統合アッセイデバイスは、以下の１つ以上も含む：検出システム（例えば、電極および／または関係するエレクトロニクスを含むボルタンメトリーシステム）、緩衝液および／またはフラッシング流体を供給する１つ以上のリザーバ、サンプルアプリケーションウェルおよび／または注入口、（ポンプ、リザーバフロー切り替え、検出器およびシグナル分析システムなどの制御のための）コンピュータコントローラ。

【００９６】

特に好ましい実施形態では、統合アッセイデバイスはチャネルを「着脱可能な」ユニットに備える。従って例えば、付随デバイスから容易に挿入および着脱できるキャピラリーがモジュールにチャネルとして設けられ得る場合、それによりデバイスは分析物の異なるセットのアッセイで容易に稼働できるようになる。

【００９７】

デバイスで使用されるチャネルが管（例えば、キャピラリー電気泳動管）である場合、従来のキャピラリー電気泳動デバイスは、多くの、本発明に従った「統合」アッセイデバイスのための付随的配管、サンプル取扱い構成要素および送給構成要素およびコンピュータコントローラを備えている。多様な分析物の検出および／または定量に良好に適する統合アッセイデバイスを提供するために、本発明に従った検出器（例えば、シヌソイドボルタンメトリー検出器）および関係するエレクトロニクスのかなり単純な導入／追加以外ほとんど必要ではない。

【００９８】

（ＩＶ．アッセイの実行）

一般に、アッセイは、固定された結合相手を有するチャネルにサンプルを導入することによって実行される。サンプルは、それぞれの結合相手が、サンプルに存在し得る標的分析物と特異的に結合できるようにする条件下に好適に保たれる。その後、サンプルは、一般に結合した分析物の放出を助成する緩衝液の導入によって、チャネルからフラッシュされる。その後放出された分析物は下流の検出ポイントで検出され、そして分析物の正体が放出から検出までの時間によって決定される。

【００９９】

（Ａ）サンプルの調製

事実上あらゆサンプルが、この有利なデバイスおよび方法を用いて分析できる。しかし、好ましい実施形態では、サンプルは生物学的サンプルである。用語「生物学的サンプル」は、本明細書中で使用されるように、生物体または生物体の構成要素（例えば細胞）から得られるサンプルをいう。サンプルはあらゆる生物学的組織または流体のものとし得る。多くの場合、サンプルは、患者に由来するサンプルである「臨床的サンプル」である。そのようなサンプルは、以下に限らないが、喀痰、脳脊髄液、血液、血液画分（例えば血清、血漿）、血球（例えば白血球）、組織または細針生検サンプル、尿、腹水および精水、またはそれらに由来する細胞を含む。また、生物学的サンプルは、組織学上の目的で得られた凍結切片といった組織切片も含み得る。

【０１００】

生物学的サンプル（例えば血清）は直接分析されるでもよいし、それらは本発明のアッセイでの使用前に何らかの調製に供されてもよい。そのような調製は、以下に限らないが、水または適切な緩衝液におけるサンプルの懸濁／希釈または、例えば巨心分離などによる細胞残屑の除去、または分析前のサンプルの特定の画分の選択を含み得る。

【０１０１】

（Ｂ）システムへのサンプルの送給

サンプルは、当業者に周知の標準方法に従って本発明のデバイスに導入できる。従って例えば、サンプルは、高圧液体クロマトグラフィーシステムにおいて使用されるもののような注入口を通じてチャネルに導入することができる。別の実施形態では、サンプルはチャネルと追絡しているサンプルウェルに適用できる。さらに別の実施形態において、サンプ

ルはチャネル内にポンプ送給され得る。サンプルをチャネルに導入する方法は周知であり、キャピラリー電気泳動法およびクロマトグラフィーの技術において標準である。

【0102】

(C)結合条件)

一旦チャネルに入ると、サンプルは、サンプルと結合相手との間での特異的な結合を促進する条件のもとに保たれる。結合相手と分析物との間での特異的な結合に適合する条件は、当業者に周知である。例えば、抗体と標的タンパク質との間の結合を促進するために適切な緩衝液がイムノアッセイ技術において周知である(例えば、米国特許第4,366,241号、第4,376,110号、第4,517,288号および第4,837,168号;Asai(1993)Methods in Cell Biology Volume 37:Antibodies in Cell Biology,Academic Press,Inc.New York;Stites & Terr(1991)Basic and Clinical Immunology 7th Edition参照)。同様に、核酸が相互に特異的にハイブリダイズする際の条件も当業者に周知である(前掲Tijssen(1993)参照)。特定の結合条件は、当業者に周知の標準方法に従って、結合相手と標的分析物との特定のセットについて最適化される(例えば、前掲Tijssen(1993)、米国特許第4,366,241号、第4,376,110号、第4,517,288号および第4,837,168号;Asai(1993)Methods in Cell Biology Volume 37:Antibodies in Cell Biology,Academic Press,Inc,New York:Stites & Terr(1991)Basic and Clinical Immunology 7th Edition参照)。

【0103】

(D)放出条件)

サンプル中の分析物がチャネルに固定した結合相手と特異的に結合された後、それらは放出される。放出は、結合相手/分析物複合体を緩衝液と接触させることによってまたは、結合相手/分析物の相互作用を破壊する温度条件によって好適に行われる。そのような合は、特定の分析物/結合相手のペアに応じて、高温、変性剤(例えば、尿素、ホルムアミドなど)、高または低pH、高または低塩類、および様々なカオトロピック試薬(例えば塩酸グアニジン)の使用によって破壊され得る。

【0104】

(E)チャネル内の分析物/流れ)

サンプルおよび/またはキャリヤー/緩衝液流体は、標準の方法に従ってチャネルへ導入し、かつ/または、チャネル内を移動させることができる。例えば、流体は「リザーバ」からの単純な重力給送によってチャネル内へ導入され、そして移動され得る。あるいはまた、流体は、ガス圧力、または多様な適切なポンプ(例えば、ぜん動ポンプ、計量ポンプなど)のうちのいずれかによって生じた液圧、変形可能なチャンバ/ダイヤフラムへの圧力などによってチャネル内を移動され得る。また分析物も、電気浸透方法によってチャネル内を移動され得る。

【0105】

(F)検出)

上述の通り、分析物検出は、上述のような当業者に周知の多くの方法のいずれかによることができる。好ましい実施形態において、電気化学的検出方法が使用され、最も好ましい実施形態では、検出はシヌソイドボルタンメトリーによっている。

【0106】

シヌソイドボルタンメトリーを実行するためのプロトコルは、既に記載されている(Singhal et al.(1997)Anal.Chem.69:4828-4832;および米国特許第5,650,061号)。簡単にいえば、2Hz、0.7Vp-p、+0.35V直流オフセットの正弦波が、ソフトウェアプログラムを用いてデジタル生成される。この正弦波は、個電極への印加電位として働く。電極からの電流応答は、単一の

溶出ランの全長の間、リアルタイムでソフトウェアによって収集される。この時間ドメイン電流応答はその後、高速フーリエ変換によって周波数ドメインに変換される。周波数スペクトルを分析するためのプロトコルは既述されている（Ｓｉｎｇｈａｌ　ｅｔ　ａｌ．（１９９７）Ａｎａｌ．Ｃｈｅｍ．６９：１６６２－１６６８）。分析物に対応するスペクトルは、既述の通り（前掲Ｓｉｎｇｈａｌ　ｅｔ　ａｌ．（１９９７））バックグラウンドサブトラクションおよびデジタル位相ロックの後に得られる。

【０１０７】

（Ｖ．複数分析物検出用キット）

１実施形態において、本発明は、サンプル中の多数の分析物の存在または不在を識別するまたは定量化するためにスクリーニングするキットを提供する。キットは、本明細書中に示される通り各自の表面に固定された各種結合相手を保持する本発明のチャネルを含む。チャネルは、例えば、本明細書中に説明した通り、電気化学的検出器（例えば、シヌソイドボルタンメトリー）回路、サンプルの管理およびチャネル内の流体の流れの維持のための適切な配管、およびサンプルの適用、流体の流れおよび信号出力の分析の制御のためのコンピュータ制御システムを備えるデバイスといった、一体型アッセイデバイスへの単純かつ迅速な組込みのために設計され得る。キットは、本明細書中に述べたアッセイ方法での使用に適切な緩衝液ならびに他の溶液および標準物質をさらに含むことができる。

【０１０８】

さらに、キットは、本発明の方法を実施するための指示（すなわち、プロトコル）を含む教材を含み得る。教材は一般に、書面または印刷物を含むが、それらはそうしたものに制限されない。そのような指示を格納し、それらをエンドユーザに伝達することができるあらゆる媒体が本発明によって考慮されている。そのような媒体は、以下に限らないが、電子格納媒体（例えば、磁気ディスク、テープ、カートリッジ、チップ）、光学式媒体（例えばＣＤ－ＲＯＭ）などを含む。そうした媒体は、そのような教材を提供するインターネットサイトへのアドレスを含み得る。

【０１０９】

（実施例）

以下の実施例は、本願発明を例示するために提示するものであり、限定するためではない。

【０１１０】

（実施例１）

（ＤＮＡハイブリダイゼーションのナノリットル体積の電気化学的検出）

（材料および方法）

（試薬）

使用する水は脱イオン化した後、Ｍｉｌｌｉ－Ｑ浄水システム（Ｍｉｌｌｉｐｏｒｅ　Ｃｏｒｐ．，Ｂｅｄｆｏｒｄ，Ｍａｓｓ．）を通過させた。結核（ＴＢ）およびヒト免疫不全ウイルス（ＨＩＶ）の同定に特異的なビオチン化ＤＮＡプローブおよびｃＤＮＡ標的を、Ｇｅｎｅｍｅｄ　Ｓｙｎｔｈｅｓｉｓ，Ｉｎｃ．（Ｓａｎ　Ｆｒａｎｃｉｓｃｏ，Ｃａｌｉｆ．）を通じて特注合成した（表１）。ＤＮＡプローブ溶液は、脱イオン水に溶解させたＤＮＡプローブの１００μｇ／ｍｌ溶液をＤＮＡ結合溶液（Ｐｉｅｒｃｅ　Ｃｈｅｍｉｃａｌｓ，ＣＡ）との１：１混合物に希釈することによって作製した。この結合溶液は、蒸水および静電相互作用によって重合化表面にＤＮＡを結合するのを容易にする。溶融シリカキャピラリー（Ｐｏｌｙｍｉｃｒｏｎ　Ｔｅｃｈｎｏｌｏｇｉｅｓ，Ｉｎｃ．，ＡＺ）を、キャピラリーバイオセンサを作製するために使用した。これらのキャピラリーをアセトンでフラッシュせず、キャピラリー表面に何らかの誘導体化を行う前に乾燥させた。

【０１１１】

（ＤＮＡプローブのキャピラリー誘導体化および固定化）

溶融シリカキャピラリー（内径５０μｍ×外径１５０μｍ、長さ１ｍ）をバイオセンサに使用した。キャピラリーを、有機被覆で溶融シリカ表面を被覆するために、エポキシ樹脂

（Ｅｐｏｔｅｋ３５０）の薄層で被覆した。表面の有機被覆は、キャピラリーの壁のＤＮＡ吸着を最小限にするだけでなく、ＤＮＡプローブが直接固定化され得る整合化表面を付与する。キャピラリー表面をエポキシ樹脂で被覆するプロトコルは、Ｌｉｕら（Ｌｉｕ ｅｔ ａｌ．（１９９６）Ｊ．Ｃｈｒｏｍａｔｏｇｒ．７２３：１５７－１６７）が正確に説明した通りであった。簡単にいえば、キャピラリーを、最初にアセトンで１５分間すすぎ洗いした後、２０ｐｓｉの窒素圧下で１時間、１００℃でオーブンにて乾燥した。エポキシ樹脂３１４ＮＤ（Ｅｐｏ－Ｔｅｋ，Ｂｉｌｌｅｒｉｃａ，ＭＡ）を、エポキシ樹脂混合物のアセトン溶液を吸引することによってキャピラリー表面に動的に被覆した。残留溶媒を、室温で３０分間、窒素でフラッシュすることによってエポキシ樹脂被覆キャピラリーから除去した。エポキシ樹脂被覆は８０℃で３０分間、さらに２０ｐｓｉの窒素圧下で１５０℃で２時間、架橋した。被覆したキャピラリーを、使用前に３０分間緩衝液で洗浄した。

【０１１２】

次いで、エポキシ樹脂被覆キャピラリーの１ｃｍセクションを、特定のＤＮＡプローブ溶液でフラッシュした。ＤＮＡプローブ溶液を一晩キャピラリー片と反応させて、ＤＮＡプローブを疎水および静電相互作用によってキャピラリー壁と結合させた。他のＤＮＡプローブを、同様の１ｃｍ長の被覆キャピラリー片に同様にして固定した。プローブがキャピラリー壁に固定化されると、それらのハイブリダイゼーション領域を脱イオン水ですすいだ後、キャピラリーバイオセンサに組み立てる準備が整った。これらのハイブリダイゼーション領域を、入口から第１のプローブ（ＴＢプローブ）までの距離が約２５ｃｍであり、２つのプローブが１５ｃｍ離れた、２つの異なる位置で「分離カラム」にエポキシ樹脂で接着した。これにより、第２のプローブ（ＨＩＶプローブ）から検出器まで約６０ｃｍの距離が残された。キャピラリーの異なるセグメントを、やはり各々約１ｃｍの長さであるスリーブ（１８０×３６０μｍキャピラリーセクション）にキャピラリーをエポキシ樹脂で接着することによってともに連結した。キャピラリーバイオセンサの全長は約１ｍであった。

【０１１３】

（ＤＮＡ標的のハイブリダイゼーション、溶出および検出）

キャピラリーを、商業用キャピラリー電気泳動デバイス（Ｂｉｏｒａｄ Ｉｎｓｔｒｕｍｅｎｔｓ Ｉｎｃ，Ｈｅｒｃｕｌｅｓ，ＣＡ）に取り付け、このデバイスをその加圧フローおよびオートサンプラー機能のために使用した。これらのＤＮＡプローブに相補的標的を高ストリンジェンシーでハイブリダイズするために使用されるプロトコルは、文献に広範に記載されている。この実験に使用された特定のプロトコルは次の通りである。

【０１１４】

最初に、ｃＤＮＡ標的をプローブと選択的に結合させるために、キャピラリーを、プレハイブリダイゼーション緩衝液（０．７５Ｍ ＮａＣｌ、７５ｍＭクエン酸ナトリウム、ｐＨ＝７．０、０．１％Ｎ－ラクトイルサルコシン、０．０２％ＳＤＳ、５０％ホルムアミド中、４０℃）でフラッシュした。ＴＢおよびＨＩＶ標的両方のＤＮＡ標的溶液を、プレハイブリダイゼーション緩衝液に溶解し、フラッシュし、キャピラリーにて約３０分間インキュベートし、表面固定化プローブの完全なハイブリダイゼーションおよび飽和を得た。

【０１１５】

次いで、余剰標的溶液をハイブリダイゼーション緩衝液（０．３Ｍ ＮａＣｌ、３０ｍＭクエン酸ナトリウム、ｐＨ＝７．０、０．１％ＳＤＳ）ですすぎ出した。その後ストリンジェント洗浄を、いずれの非特異的結合したＤＮＡ標的も除去するために、ストリンジェント洗浄緩衝液（７５ｍＭ ＮａＣｌ、７．５ｍＭクエン酸ナトリウム、ｐＨ＝７．０、０．１％ＳＤＳ、４０℃）により行った。このストリンジェント洗浄により、他のすべてのものはこれらのストリンジェント条件のもとで洗い出されるので、完全に相補的ＤＮＡ標的だけがキャピラリーバイオセンサ内部に残されることが保証された。

【０１１６】

次いで、キャピラリーを、（界面活性剤の存在のために）銅電極と適合しない高ストリンジェンシー洗浄緩衝液をすすぎ出すために、電気化学的洗浄緩衝液（８９ｍＭ　ＴＲＩＳ、８９ｍＭホウ酸および１ｍＭ　ＥＤＴＡ、ｐＨ＝１０）で充填した。

【０１１７】

一旦、キャピラリーを電気化学的洗浄緩衝液で充填すると、銅電極はバイオセンサキャピラリー出口に保たれた。電極を、ツーパート機械加工設計（ｔｗｏ－ｐａｒｔ　ｍａｃｈｉｎｅｄ　ｄｅｓｉｇｎ）（Ｋｕｈｒ（１９９３）米国特許第５，６５０，０６１号）によりキャピラリー出口と自動的に整列させた。次いで、キャピラリーを、溶出緩衝液（８９ｍＭ　Ｔｒｉｓ、８９ｍＭホウ酸および１ｍＭ　ＥＤＴＡ、ｐＨ＝１１）を（１００ｐｓｉで）迅速に満たし、室温で３０分間インキュベートした。溶出緩衝液は、ハイブリダイズしたＤＮＡ標的の変性を促進し、それによって、特異的な位置でキャピラリー内部の溶液中にオリゴマーを放出した。

【０１１８】

次いで、脱ハイブリダイズされた標的ＤＮＡを含む溶出緩衝液を、約５ｐｓｉで加圧誘起フローを用いて一定の速度でポンプ送給し、それによってそれらが緩衝液とともに移動する際に放出されたＤＮＡ標的を溶出した。ＤＮＡ標的オリゴマーが検出器を通過して流れると、ＤＮＡは銅電極で電気触媒作用で酸化され、それにより、既述の通り（米国特許第５，６５０，０６１号参照）シヌソイドボルタンメトリーを用いて検出され得る信号を発生させた。その後ＤＮＡの各個別のゾーンが、ＤＮＡが検出器を通過して移動する際に出口の銅電極で検出された。

【０１１９】

（電気化学的検出）

直径４０ミクロンの銅微小電極を、５ｃｍ、５０×３６０μｍ溶融シリカキャピラリーの内側に製作した。キャピラリーを、シリンジを用いてガリウムで満たした。次に、小さい長さの銅ワイヤを一端でキャピラリーに挿入した後、５分のエポキシ樹脂接着によって適所に密封した。別のワイヤを、キャピラリーの後端から挿入して、銅ワイヤとの電気接続を与えた。キャピラリー内部のガリウムが２個のワイヤ間の電気接続を与えた。これらのキャピラリー微小電極は、非常に丈夫であり、研磨後に再使用可能である。これらの電極を、６００粒度のサンドペーパーを用いた手による研磨以外、いかなる形態でも前処理しなかった。

【０１２０】

脱ハイブリダイズされたＤＮＡ標的を、それがキャピラリーから溶出した場合に銅微小電極で検出するために、シヌソイドボルタンメトリーを使用した。シヌソイドボルタンメトリーを実行するためのプロトコルは既述されている（Ｓｉｎｇｈａｌ　ｅｔ　ａｌ．（１９９７）Ａｎａｌ．Ｃｈｅｍ．６９：４８２８－４８３２；米国特許第５，６５０，０６１号）。簡単にいえば、２Ｈｚ、０．７Ｖｐ－ｐ、＋０．３５Ｖ直流オフセットの正弦波を、企業内ソフトウェアプログラムを用いてデジタル生成した。この正弦波は、銅電極への印加電位として働いた。電極からの電流応答を、単一の溶出ランの全長の間、リアルタイムでソフトウェアによって収集した。次いで、この時間ドメイン電流応答を、高速フーリエ変換によって周波数ドメインに変換した。周波数スペクトルを分析するためのプロトコルは既述されている（Ｓｉｎｇｈａｌ　ｅｔ　ａｌ．（１９９７）Ａｎａｌ．Ｃｈｅｍ．６９：１６６２－１６６８）。分析物に対応するスペクトルは、既述の通り（前掲Ｓｉｎｇｈａｌ　ｅｔ　ａｌ．（１９９７））バックグラウンドサブトラクションおよびデジタル位相ロックの後に得られた。

【０１２１】

（結果および考察）

ＤＮＡハイブリダイゼーションの低量、直接的な検出は、疾患の指標としてＤＮＡが臨床的に重要であるために望ましい。一旦、特定のヌクレオチド配列が所定のマーカー（例えば、感染因子、遺伝形質、腫瘍タイプ）と特有にまたは識別可能に関係づけられることが示されれば、その配列は、大量に合成され、その特定の配列が存在するかどうかを決定す

るために他の供給源から核酸のプローブとして使用することができる。ハイブリダイゼーションに基づくＤＮＡアッセイは多くの異なる用途のために開発されており、多くの場合、存在するＤＮＡを完全にフィンガープリントし、そして同定するためにすべてのサンプルについて複数の試験が実行される必要がある。

【０１２２】

周波数ドメインボルタンメトリー検出技術であるシヌソイドボルタンメトリーは、標識の検出に使用されるものと同様の実験条件の下で核酸を検出するために使用できる。ヌクレオチドも核酸塩基にアミン部分を含み、そしてそれらも銅表面で電気活性であるので、ヌクレオチドの何らかの信号が糖主鎖によるものとは別にそれらの塩基によって寄与され得ることが可能であった。

【０１２３】

非誘導体化ＤＮＡの検出は、あらゆるサンプル取扱損失および汚染問題を回避するために非常に望ましい。電気化学的検出は、高感度な検出器としてその能力を犠牲にすることなく容易に小型化できる（ナノリットルからピコリットル容量で作業し得る）ことから、ＤＮＡ分析の鍵してサンプルの限られた場合に特に適する。

【０１２４】

このキャピラリーバイオセンサの開発において、ＤＮＡの特定の配列を、連続する微小流体チャネル（すなわち、溶融シリカキャピラリー）内部の異なる領域に固定化した。２０ｎＬのサンプル体積に一致する内径５０μｍキャピラリーの１ｃｍセクションを、センサの認識領域を付与するために使用した。サンプルを、個々の領域を通して順次的にポンプ送給し、そこで、適切なＤＮＡ標的（存在すれば）が個々の固定化ＤＮＡプローブと独立して結合し得る。一旦、サンプルが個々の固定化された標的と相互作用する機会を持てば、それはキャピラリーから溶出され、キャピラリー全体は一連のストリンジェント洗浄で洗浄されて、それによって、材料を汚染するあらゆる可能性が排除された。次いで、固定化されたプローブの各領域と結合したままの標的ＤＮＡを、空間的にコードされた様式で溶出した。

【０１２５】

図１は、単一の実験において複数のハイブリダイゼーション事象を観察する可能性を与えるためにこの設計において使用された基本的アプローチを示す。ゾーン１および２は、ＴＢおよびＨＩＶのＤＮＡプローブがそれぞれ固定された固定化ゾーンである。これらのゾーンは後に、ＤＮＡ標的を含有するサンプルの１回だけの注入を使用するために、単一のキャピラリーシステムを作製するために組み合わされた。極めて高いストリンジェンシーでより複雑なサンプル（すなわち、タンパク質、他の細胞除などのような多数の他の生体分子を含んでいる臨床的サンプル）を洗浄するために必要な試薬は、キャピラリーの先頭のリザーバから加圧誘起された流れによって導入することができる。微細小電極は、キャピラリーの出口端に配備され、それは、電極とのキャピラリーの自動整列を可能にする微械加工ツーバートシステムを用いて配置される（Ｋｕｈｒらの米国特許第５，６５０，０６１号）。従って、システムは組み合わせるのが非常に容易であり、一旦稼働すると頑強である。

【０１２６】

特異的ハイブリダイゼーション、洗浄および、変性した標的オリゴヌクレオチドの溶出を実行するために使用される工程の順序は、図２に示されている。同様な工程は、それらの相補的プローブへのＤＮＡ標的のあらゆる種類のストリンジェントなハイブリダイゼーションに使用することができる。このスキームにおいて、

１）ハイブリダイゼーションは、キャピラリー壁、または標的分析物に対して完全な相補体ではないプローブへの標的のあらゆる非特異的な結合を回避するために、ストリンジェント条件のもとで実行される。その結果、ＴＢ標的（ＴＢをコードするＤＮＡに特徴的な配列を有するオリゴマー（ゾーン１）は、固定化されたＴＢプローブ（相補的配列）にハイブリダイズするだけであり、ＨＩＶ標的は、ストリンジェント条件下で固定化されたＨＩＶプローブ（ゾーン２）にハイブリダイズするだけである。これらのゾーンは空間的に

隔離しており、ストリンジェント洗浄は、各ゾーンからだけでなく、それらのゾーンを分離するキャピラリーからも、全部の干渉成分を除去する。

【０１２７】

２）溶出緩衝液（ＴＢＥ、ｐＨ＝１１）による最終洗浄は、ハイブリダイズした相補的核酸を同時に変性させ、それによって、結合したＤＮＡ標的をキャピラリーの固定化プローブに直接隣接する溶液に放出する。これらの２つの標的の空間的選択性は保たれる。なぜならその緩衝液は（脱ハイブリダイゼーションが生じ得るよりもずっと高速な時間スケールで）適所に迅速に移動し、そしてキャピラリー内の流れは停止し、変性プロセスは３０分のインキュベート後に完了するからである。

【０１２８】

３）最後に、「自由な」空間的に分離した標的ＤＮＡオリゴマーを含有する溶液が溶出される。その２つの標的を含んでいるゾーンが空間的に別個であるので、それらは、異なる時間に出口に配置された銅電極を過ぎて流れる。図３に示されたスキームは、溶出しているＤＮＡ標的を検出するその局面を例示している。検出器における各標的の溶出時間はその正体を示し、それによりＤＮＡハイブリダイゼーションの部位をコード化する。

【０１２９】

固定化ＤＮＡプローブの１ｃｍゾーンによるキャピラリーバイオセンサを用いたＨＩＶ標的ＤＮＡの検出は、図４に示されている。１０μｇ／ｍｌの合成ＨＩＶ標的の１００μＬを含有するサンプルを、ＨＩＶプローブが固定化されたキャピラリーバイオセンサ内を通してフラッシュした。サンプルのＨＩＶオリゴヌクレオチド標的の検出を可能にするために、図２に記載した工程の順序に従った。本来、その順序は電気化学的洗浄緩衝液（８９ｍＭ　ＴＲＩＳ、８９ｍＭホウ酸および１ｍＭ　ＥＤＴＡ、ｐＨ＝１０）を含まなかった。これを、溶出緩衝液が銅電極に攻撃する時に観察される人工産物を最小限にするために添加した。この緩衝液のｐＨは重要である。なぜなら、過度に高いｐＨは標的ＤＮＡの脱ハイブリダイゼーションにつながり、信号の損失をもたらす一方、過度に低いｐＨは溶出緩衝液が検出器に到達した時に大きな人工産物を結果として生じるからである。

【０１３０】

図４に示すように、シヌソイドボルタンメトリーで得られた信号は、溶出緩衝液での脱ハイブリダイゼーションの後にＤＮＡ標的の溶出を実証する。ブランク溶液の溶出は、信号が非常に安定しいることを示すが、単一プローブシステムによるＨＩＶ標的の結合の特異性を評価することは困難である。従って、この種の検出はＤＮＡ試験において偽陽性をもたらす可能性がある。

【０１３１】

複数プローブシステムは、核酸サンプルの並行処理の問題に取り組むことができるだけでなく、その固有の設計において非特異的なハイブリダイゼーションに対する内部基準を付与する。非特異的なハイブリダイゼーションが所与のサンプルで生起した場合、それは複数プローブシステムにおいて複数のピークを与えるであろう。これは、単一の注入した標的について単一のピークが検出されるまで、よりいっそうストリンジェントなハイブリダイゼーションプロトコルの必要性を直接示すことになる。このシステムのハイブリダイゼーションの特異性は、図５（Ａ）に例証されており、ＴＢおよびＨＩＶの特異的標的のハイブリダイゼーションの検出が同一サンプルにおいて同時に存在する。サンプルは各ＤＮＡプローブと１回だけ相互作用させられたが、２つの標的は１回のランで同時に検出することができる。２つのゾーンに関する移動時間は、図５（Ｂ）および５（Ｃ）にそれぞれ示されたＴＢおよびＨＩＶ標的の内部基準と合致する。従ってこれは、２つの標的が同時に検出できるだけではなく、使用されているハイブリダイゼーション条件のもとで生起するいかなる非特異的なハイブリダイゼーションが存在しないことも示している。そうでなければ、内部基準ランは１つではなく２つのピークを示したであろう（すなわち、ＴＢ特異的標的は自己の完全に相補的なプローブおよびＨＩＶ特異的プローブに対しハイブリダイズしたであろうし、ＨＩＶ特異的標的についても同様である）。従って、図５（Ａ）における２つのピークの検出は、合成のＴＢおよびＨＩＶ特異的標的の検出を同時にはっき

りと示しており、非特異的なハイブリダイゼーションの不在を例証しており、あらゆる偽陽性の結果の発生の見込みを低減させる。

【０１３２】

ハイブリダイゼーションによるＤＮＡシークエンシングは、サンプル（例えば、標的）ＤＮＡ分子の固定化プローブＤＮＡへのハイブリダイゼーションによって与えられる分子認識に依存する。好ましいプローブオリゴヌクレオチドは、長さが少なくとも約７のヌクレオチドであり、より好ましくは長さが少なくとも約１０のヌクレオチドであり、さらに好ましくは長さが少なくとも１５または２０のヌクレオチドであり、最も好ましくは長さが少なくとも３０、４０または５０のヌクレオチドである。このプローブは、標的の少なくとも１領域に相補的である既知の配列を有する。多数の異なるアッセイ形式が存在するが、プローブは代表的に、ニトロセルロース、アガロース、プラスチックまたは、サンプルと接触して置かれ、非認識ＤＮＡをきれいに洗浄した後、含量についてアッセイすることができる、他の不活性基質に固定化される。ハイブリダイズしたＤＮＡのアッセイは、本明細書中に記載したシステムにおいて、ＤＮＡの変性、キャピラリーまたはチャネルからの溶離、銅微小電極でのＳＶによる検出を伴って遂行できる。

【０１３３】

（結論）

新しいキャピラリーベースのＤＮＡバイオセンサを、複数のＤＮＡオリゴマーを同時に検出することができる直接的な電気化学的検出を利用して開発した。この検出スキームは、キャピラリー表面の様々な位置に固定化された様々なＤＮＡプローブにより、サンプル中のＤＮＡ標的のフローコード化ハイブリダイゼーションアッセイを利用した。各種タイプのＤＮＡ標的の同時ハイブリダイゼーションは、それらが溶離されると、シヌソイドボルタンメトリーを使うことによる銅電極におけるそれらの標的の直接検出によって福足される。疾患特異的なオリゴヌクレオチド配列のそのような並行的かつ生の検出は、頑強で、耐久性がありかつ安価な複数疾患ＤＮＡセンサへの道を開くことができる。従って、それは、実行がオペレータ集約的で高価である各種光学式検出スキームに基づく既存のＤＮＡセンサに伴う問題を回避する。

【０１３４】

（実施例２）

（シヌソイドボルタンメトリーによるアミノ酸およびペプチドの高感度かつ選択的検出）

（実験パラメータ）

（試薬）

使用する水は脱イオン化した後、Ｍｉｌｌｉ－Ｑ浄水システム（Ｍｉｌｌｉｐｏｒｅ Ｃｏｒｐ．，Ｂｅｄｆｏｒｄ，Ｍａｓｓ．）を通過させた。アミノ酸およびインスリン（９８～９９％、Ｓｉｇｍａ Ｃｈｅｍｉｃａｌ Ｃｏｒｐ．，Ｓｔ．Ｌｏｕｉｓ，Ｍｏ．）、および残りのペプチド（Ｐｅｎｉｎｓｕｌａ Ｌａｂｏｒａｔｏｒｉｅｓ，Ｉｎｃ．，Ｓａｎ Ｃａｒｌｏｓ，ＣＡ）を、受領したまま使用した。全ての実験を、泳動電解質として０．１０水酸化ナトリウム（Ａ．Ｃ．Ｓ等級、Ｆｉｓｈｅｒ Ｓｃｉｅｎｔｉｆｉｃ，Ｆａｉｒ Ｌａｗｎ ＮＪ）により行った。０．１０Ｍの原液を脱イオン水にて調製した。以後の希釈を泳動電解質を用いて行った。

【０１３５】

（銅微小電極）

銅微小電極は、最初に微小電極プラー（Ｍｏｄｅｌ ＰＥ－２，Ｎａｒｉｓｈｉｇｅ，Ｔｏｋｙｏ Ｊａｐａｎ）によりガラスキャピラリーを引張ることによって作製した。その後、顕微鏡下でキャピラリーの端をスカルペルで切り取った。その後、直径２０μｍの銅線（９９．９９％、Ｇｏｏｄｆｅｌｌｏｗ，Ｃａｍｂｒｉｄｇｅ，Ｅｎｇｌａｎｄ）を、新しく切り取られた端部に挿入し、エポキシ樹脂で密封した（Ｅｐｏｘｙ Ｔｅｃｈｎｏｌｏｇｙ，Ｂｉｌｌｅｒｉｃａ，Ｍａｓｓａｃｈｕｓｅｔｔｓ）。電極は、ダイヤモンド研磨ホイールで研磨し、脱イオン水での超音波処理によって清浄した。銅線との電気接続を作るために、キャピラリーの後端をガリウム（Ｓｉｇｍａ Ｃｈｅｍｉｃ

ａｌ Ｃｏ.）で充填し、直径１５０μｍ銅線をガリウムに挿入した。代替として、キャピラリーの後端をエポキシ樹脂で充填し、より大径の銅線を、それが２０μｍワイヤと物理的に接触するまで、エポキシ樹脂充填キャピラリーに入れた。いかなる電気化学的前処理も実行せず、電極は、実験条件下で約１時間の間、または安定した応答が観察されるまで、安定化させた。

【０１３６】

（電気化学的計測および実験条件）

フローセルはプレキシガラスで構成し、管は拡散的広がりが回避されるように整合した。サンプルプラグの導入は、電磁弁により制御される空気式アクチュエータによって制御した。流速は、フローセルの１９ｃｍ上に緩衝液リザーバを保つことによって重力流れにより維持した。流速を０．５ｍｌ／分であると決定し、サンプルの体積を注入の流速および長さから決定した。注入時間は、電極が分析物の完全な濃度を見るように決定した。

【０１３７】

報告された実験の条件をここで説明する。アミノ酸およびペプチドの場合、２Ｈｚ正弦波（０～６９０ｍＶ対Ａｇ／ＡｇＣｌ）が、Ｌａｂｖｉｅｗ（Ｎａｔｉｏｎａｌ Ｉｎｓｔｒｕｍｅｎｔｓ，Ａｕｓｔｉｎ，Ｔｅｘ.）で著者によって書かれたソフトウェアにより適用した。波形は、基本周波数の３倍（６Ｈｚ）の３ｄｂ点によりｃｙｂｅｒａｍｐ（Ｍｏｄｅｌ ３８０，Ａｘｏｎ Ｉｎｓｔｒｕｍｅｎｔｓ Ｉｎｃ.，Ｆｏｓｔｅｒ Ｃｉｔｙ，ＣＡ.）を用いて４極低域遮断フィルタでろ波した。出力電流は４極低域通過フィルタでろ波した。フィルタは４０Ｈｚに設定された（観察された最大周波数の４倍、第１０高調波または２０Ｈｚ）。電流は、３００ＭＨｚ Ｐｅｎｔｉｕｍ（登録商標） ｉｉパーソナルコンピュータを用いて１６ビットアナログ－ディジタル変換器（ＰＣＩ－４４５１，Ｎａｔｉｏｎａｌ Ｉｎｓｔｒｕｍｅｎｔｓ）によりディジタルからアナログに変換した。単一のスキャンは４正弦波周期から構成された。

【０１３８】

収集された時間ドメインは、Ｌａｂｖｉｅｗソフトウェア（Ｎａｔｉｏｎａｌ Ｉｎｓｔｒｕｍｅｎｔｓ）によって周波数ドメインに変換し、ＭａｔｌａｂプログラミングツールＴｈｅ Ｍａｔｈｗｏｒｋｓ，Ｉｎｃ.，Ｅｎｇｌｅｗｏｏｄ Ｃｌｉｆｆｓ ＮＪ）を用いてさらに処理した。注入前に得られたバックグラウンドベクトルを瞬時信号電流ベクトルから引くことによって信号だけのスペクトルを得た。時間ドメインスペクトルを取得するためにディジタルロックイン増幅法を使用した。時間スペクトルは、各周波数高調波（最大第１０高調波まで）の振幅および位相角を生成するために５１２ポイントのレートでフーリエ変換した。各高調波の位相情報は、信号だけのベクトルを使用して、それをバックグラウンド減算信号ベクトルに投影することによって得た。最後に、位相分解ベクトルは、移動平均平滑化（箱形積分）を用いて低域ろ波通過した。

【０１３９】

（結果）

図６は、銅微小電極でのアルギニンのバックグラウンド減算周波数スペクトルを示す。実験は１μＭアルギニンを用いて実行した。励起信号は２Ｈｚ、０～６９０ｍＶ対Ａｇ／ＡｇＣｌの正弦波であった。５１２ポイント（全体時間＝１秒）から成る４正弦波周期からの電流を、各周波数スペクトルを生成するために使用した。三次元グラフは、第１０高調波までの、周波数（ｘ軸）、振幅（ｚ軸）および位相角（ｙ軸）情報から成っている。

【０１４０】

図７は、第５高調波（１０Ｈｚ）での１μＭアルギニンからの正弦波時間ドメイン応答を示す。この高調波は、最高の信号／雑音比および３９ｎＭの検出限界（Ｓ／Ｎ＝３）を与えた。

【０１４１】

図８は、様々なアルギニン濃度の線形ダイナミックレンジを示す。１、１０、１００および１０００μＭのアルギニン濃度をフロー注入分析システムに注入した。第５高調波（１０Ｈｚ）の振幅が、注入された４つの異なる濃度に対してプロットされている。このプロ

ットは、第５高調波での３オーダにわたる優れた直線性（Ｒ＝０．９９９７）を示す。
【０１４２】
図９は、銅微小電極でのアスパラギンおよびグルタミンの減算周波数スペクトルを示す。四角形は１０μＭアスパラギンを表し、円は１０μＭグルタミンを表している。実験条件は、図１を生成するために使用したものと同じである。
【０１４３】
図１０Ａおよび１０Ｂは、第６高調波（１２Ｈｚ）でのアスパラギンおよびグルタミンの正弦波時間ドメイン応答を示す。図１０Ａは１０μＭアスパラギンを示し、図１０Ｂは１０μＭグルタミンを示す。第６高調波は、それらの２つのアミノ酸の最適化位相角が９０°分離に最も近いものである。この高調波は、それらの２つの分析物間で最大の選択性を与える。アスパラギンの場合この高調波での検出限界（Ｓ／Ｎ＝３）は４００ｎＭであり、グルタミンの場合５００ｎＭである。
【０１４４】
図１１は、１０μＭインスリンＢ鎖のバックグラウンド減算周波数ドメインスペクトルを示す。図１と同じ条件を使用した。
【０１４５】
図１２は、第４高調波（８Ｈｚ）でのインスリンＢ鎖の正弦波時間ドメイン成分を示す。第４高調波は、最大の信号／雑音比および５００ｎＭの検出限界（Ｓ／Ｎ＝３）を与えた。
【０１４６】
図１３は、銅微小電極での黄体形成ホルモン放出ホルモン（円）およびブラジキニン（四角形）の減算周波数スペクトルを示す。
【０１４７】
図１４Ａおよび１４Ｂは、第２高調波（４Ｈｚ）でのブラジキニンおよび黄体形成ホルモン放出ホルモンの時間ドメイン応答をそれぞれ示す。
【０１４８】
図１５は、ニューロテンシン（四角形）およびＰ物質（円）のバックグラウンド減算周波数ドメイン応答をそれぞれ示す。
【０１４９】
図１６Ａおよび１６Ｂは、基本周波数（２Ｈｚ）でのニューロテンシンおよびＰ物質の時間ドメイン応答をそれぞれ示す。
【０１５０】
ここで説明した実施例および実施形態は例示目的だけのためであり、それに照らして様々な修正または変更が当業者に提起され、本願の精神および本文ならびに添付請求の範囲の内に含まれるはずであることが理解される。従って、ここに引用された全部の出版物、特許および特許出願は、すべての目的で完全な形で参照によりここに採り入れられる。
【図面の簡単な説明】
【図１】
図１は、電気化学的検出によるキャピラリーベースのＤＮＡバイオセンサの略図を示す。キャピラリーには２つの異なるプローブセクションが存在する。ＴＢ特異的プローブのプローブ１およびＨＩＶ特異的プローブのプローブ２である。ＨＰＣＥオートサンプラーは、これらの固定化されたプローブのｃＤＮＡ標的の特異的ハイブリダイゼーションに必要な様々なストリンジェント洗浄およびリンスに使用される。銅電極は、機械加工ツーパートシステムを使用してキャピラリーバイオセンサの出口に配置される。
【図２】
図２は、キャピラリーバイオセンサ内部でＤＮＡ標的のストリンジェントハイブリダイゼーションおよびアルカリ変性を実行するためのプロトコルを示す。（１）キャピラリー表面に固定化されたプローブに各種ＤＮＡ標的をハイブリダイズする。（２）その後ストリンジェント洗浄が、いずれかの非特異的吸着またはハイブリダイズしたＤＮＡを除去するために実行される。（３）最後に、キャピラリーバイオセンサから以前にハイブリダイズ

したＤＮＡ標的を溶離するためにアルカリ変性が実行される。

【図３】

図３は、アルカリ変性されたＤＮＡ標的のキャピラリーバイオセンサからの溶離、および引き続く電気化学的検出の略図を示す。電極は、自動整列を容易にするためにバイオセンサキャピラリーと同じ直径を備えるキャピラリー片の内側に製作される。電極はバイオセンサキャピラリーの出口に極めて近く（＜５μｍ）位置する。下側のトレースは、それらがバイオセンサキャピラリーから溶離する際のＤＮＡ標的の検出の略図を示す。

【図４】

図４は、キャピラリーバイオセンサおよびシヌソイドボルタンメトリー検出を用いたＨＩＶ特異的標的の検出を例示する。１０μｇ／ｍｌのＨＩＶ特異的標的が、ＨＩＶ特異的プローブだけが固定化されたキャピラリーバイオセンサ内部に流される。すべてのハイブリダイゼーション条件は本明細書中に記載の通りである。シヌソイドボルタンメトリー励起波形は０～７００ｍＶｐ－ｐで２Ｈｚであった。図示された信号は第５高調波で得られた。

【図５】

図５は、フローコード化ハイブリダイゼーションアッセイを同時に用いた複数のＤＮＡ標的の検出を示す。使用したサンプルは、ＨＩＶおよびＴＢの特異的標的のそれぞれ１０μｇ／ｍｌの濃度の１：１混合物を含んでいた。すべてのハイブリダイゼーションおよび溶離条件は、図４でのものおよび実施例１で説明したものと同じである。図示された信号は、それが検出に最良の感度を有するとわかったので、第５高調波で得られた。

【図６】

図６は、銅微小電極でのアルギニンのバックグラウンド減算周波数スペクトルを示す。三次元グラフは、第１０高調波までの、周波数（ｘ軸）、振幅（ｚ軸）および位相角（ｙ軸）情報から成っている。

【図７】

図７は、第５高調波（１０Ｈｚ）での１μＭアルギニンからの正弦波時間ドメイン応答を示す。

【図８】

図８は、様々なアルギニン濃度の線形ダイナミックレンジを示す。

【図９】

図９は、銅微小電極でのアスパラギンおよびグルタミンの減算周波数スペクトルを示す。四角形は１０μＭアスパラギンを表し、円は１０μＭグルタミンを表している。

【図１０】

図１０Ａおよび１０Ｂは、第６高調波（１２Ｈｚ）でのアスパラギンおよびグルタミンの正弦波時間ドメイン応答を示す。図１０Ａは１０μＭアスパラギンを示し、図１０Ｂは１０μＭグルタミンを示す。

【図１１】

図１１は、１０μＭインスリンＢ鎖のバックグラウンド減算周波数ドメインスペクトルを示す。

【図１２】

図１２は、第４高調波（８Ｈｚ）でのインスリンＢ鎖の正弦波時間ドメイン成分を示す。

【図１３】

図１３は、銅微小電極での黄体形成ホルモン放出ホルモン（円）およびブラジキニン（四角形）の減算周波数スペクトルを示す。

【図１４】

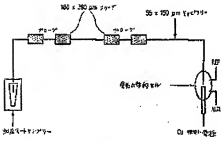
図１４Ａおよび１４Ｂは、第２高調波（４Ｈｚ）でのブラジキニンおよび黄体形成ホルモン放出ホルモンの時間ドメイン応答をそれぞれ示す。

【図１５】

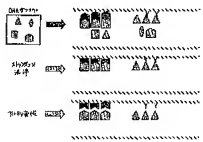
図１５は、ニューロテンシン（四角形）およびＰ物質（円）のバックグラウンド減算周波数ドメイン応答をそれぞれ示す。

【図１６】
図１６Ａおよび１６Ｂは、基本周波数（２Ｈｚ）でのニューロテンシンおよびＰ物質の時間ドメイン応答をそれぞれ示す。

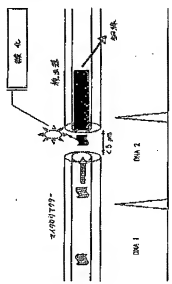
【図１】



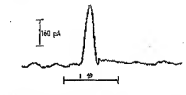
【図２】



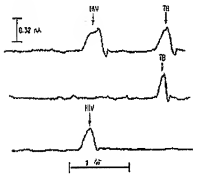
【図３】



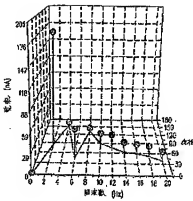
【図４】



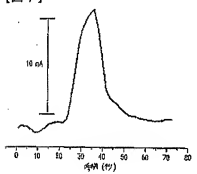
【図５】



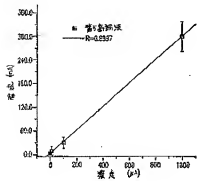
【図６】



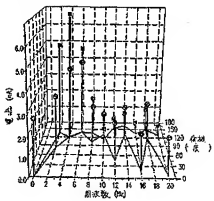
【図７】



【図８】



【図９】



【図１０】

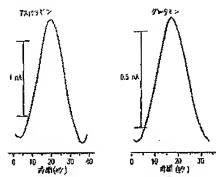
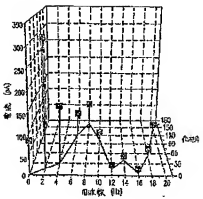
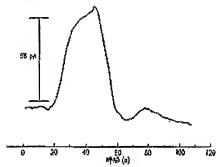


図 10A　図 10B

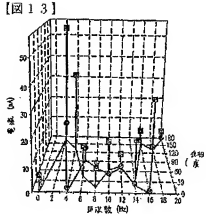
【図１１】



【図１２】



【図１３】



【図１４】

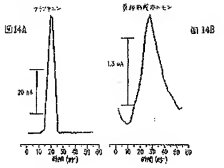
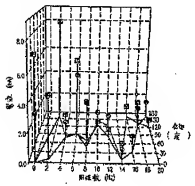


図14A　　図14B

【図１５】



【図１６】

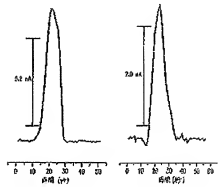


図16A　　図16B

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1]
It is an approach for detecting two or more target analyte in a sample, and these approaches are the following processes. :
i) -- this -- the process which the joint pair to each of two or more analyte provides with the channel currently fixed in it -- it is -- here -- this -- the joint pair to each of two or more analyte It is positioned in the field to which these channels differ. And this channel the cross-sectional area small enough -- having -- consequently -- this -- process; from which this analyte is spatially isolated until this analyte reaches the detecting point of this channel that is down-stream from this joint pair, when the analyte is emitted to the fluid which is flowing through this channel from two or more joint pairs
ii) Process which is a process which pours the fluid containing a sample through this channel under conditions which this target analyte that exists in this fluid combines with each of those joint pair, and codes this analyte spatially along with this channel by it,
iii) -- process; which emits this analyte to the fluid which is flowing along with this channel from this joint pair -- and
iv) Process which detects this analyte in the location which met this channel that is down-stream from this joint pair,
How to include.
[Claim 2]
The approach according to claim 1 by which the indicator of said analyte is not carried out.
[Claim 3]
The approach according to claim 1 said channel is a capillary tube.
[Claim 4]
The approach according to claim 3 said capillary tube is capillary-electrophoresis tubing.
[Claim 5]
The approach according to claim 1 said channel is a channel etched into the front face.
[Claim 6]
The approach according to claim 5 said channel is a channel etched into the glass front face.
[Claim 7]
The approach according to claim 1 by which said channel is cast.
[Claim 8]
The approach according to claim 7 by which said channel is cast with the polymer ingredient.
[Claim 9]
The approach according to claim 1 said channel has the cross section which offers less than about one Reynolds number (Re).
[Claim 10]
The approach according to claim 1 said channel has the cross-section diameter of less than about 100 micrometers.

[Claim 11]
The approach according to claim 1 said two or more target analyte contains at least three different analyte.
[Claim 12]
The approach according to claim 1 chosen from the group which said joint pair becomes from an antibody, binding protein, and a nucleic acid.
[Claim 13]
The approach according to claim 12 said joint pair is a nucleic acid.
[Claim 14]
The approach according to claim 1 the process which pours said fluid is the fluid flow guided by differential pressure.
[Claim 15]
The approach according to claim 1 the process which pours said fluid is electroendosmose fluid flow.
[Claim 16]
The approach containing the sample chosen from the group which said fluid becomes from blood, plasma, a blood serum, urine, oral cavity liquid, cerebrospinal fluid, and lymph according to claim 1.
[Claim 17]
The approach according to claim 1 said detection process contains extinction spectroscopy.
[Claim 18]
The approach according to claim 1 said detection process contains a sinusoid voltammetry.
[Claim 19]
The approach according to claim 1 said analyte is a nucleic acid and said detection process detects the target analyte by the concentration below 1x10-9M.
[Claim 20]
It is a device for detecting two or more analyte in a sample, and this device is the following. :
this -- the channel by which the joint partner to each of two or more analyte is being fixed in it -- it is -- here -- this -- this joint partner to each of two or more analyte It is positioned in the field to which these channels differ. And this channel the cross-sectional area small enough -- having -- consequently -- this -- until this analyte reaches the detecting point of having met this channel that is down-stream from this joint partner when the analyte is emitted to the fluid which is flowing through this channel from two or more joint partners The channel from which this analyte is isolated spatially; it reaches.
The detector which detects this analyte at this detecting point in this channel,
********, a device.
[Claim 21]
The device according to claim 20 said whose channel is a capillary tube.
[Claim 22]
The device according to claim 21 said whose capillary tube is capillary-electrophoresis tubing.
[Claim 23]
The device according to claim 20 said whose channel is a channel etched into the front face.
[Claim 24]
The device according to claim 23 said whose channel is a channel etched into the glass front face.
[Claim 25]
The device according to claim 20 with which said channel has the cross-sectional area which offers less than about one Reynolds number (Re).
[Claim 26]
The device according to claim 20 with which said channel has the cross-section diameter of less than about 100 micrometers.
[Claim 27]
The device according to claim 20 with which said two or more target analyte contains at least three different analyte.
[Claim 28]

The device according to claim 20 chosen from the group which said joint partner becomes from an antibody, binding protein, and a nucleic acid.
[Claim 29]
The device according to claim 28 said whose joint partner is a nucleic acid.
[Claim 30]
The device according to claim 20 with which said detector is equipped with an absorption spectrometry meter.
[Claim 31]
The device according to claim 20 with which said detector is equipped with a sinusoid voltameter.
[Claim 32]
It is a kit for detection of two or more target analyte in a fluid. This kit this -- the channel by which the joint partner to each of two or more analyte is being fixed in it -- having -- here -- this -- this joint partner to each of two or more analyte It is positioned in the field to which these channels differ. And this channel the cross-sectional area small enough -- having -- consequently -- this -- when the analyte is emitted to the fluid which is flowing through this channel from two or more joint partners, this analyte is spatially isolated until this analyte reaches the detecting point of having met this channel that is down-stream from this joint partner
Kit.
[Claim 33]
The kit according to claim 32 with which said kit is equipped with said two or more channels.
[Claim 34]
The kit according to claim 33 with which each of said channel containing said two or more channels has collection of a joint partner's proper.
[Claim 35]
The kit according to claim 33 said whose channel is a capillary tube.
[Claim 36]
The kit according to claim 35 said whose capillary tube is capillary-electrophoresis tubing.
[Claim 37]
The kit according to claim 33 said whose channel is a channel etched into the front face.
[Claim 38]
The kit according to claim 37 said whose channel is a channel etched into the glass front face.
[Claim 39]
The kit according to claim 33 with which said channel has the cross section which offers less than about one Reynolds number (Re).
[Claim 40]
The kit according to claim 33 with which said channel has the cross-section diameter of less than about 100 micrometers.
[Claim 41]
The kit according to claim 33 with which said channel contains the joint partner of at least three different kinds.
[Claim 42]
The kit according to claim 33 chosen from the group which said joint partner becomes from an antibody, binding protein, and a nucleic acid.
[Claim 43]
The kit according to claim 42 said whose joint partner is a nucleic acid.

[Translation done.]

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
(Cross-reference of related application)
This application asserts the United States patent application 09th of application / priority of No. 358,204 for all the purpose on July 21, 1999 when the whole is used as reference into this specification.
[0002]
(Statement about the right of invention performed under federal support researches and developments)
This research was supported by the National Institute of Health (GM 44112-01A1) and the UC BioSTAR project. The American government may have a fixed right in this invention.
[0003]
(Field of invention)
This invention relates to the field of a diagnosis. This invention provides a detail with the device and approach which do not need use of an indicator or an indicator attachment process, though quick detection and/or the quick quantum of two or more analyte are made possible.
[0004]
(Background of invention)
Immunoassay and nucleic-acid hybridization chemistry perform the illness diagnosis which detects a genetic defect, and are quickly developed towards the target to perform prognostic evaluation (SosnowskiProc(s)(1997).Natl.Acad.Sci.USA, 94:1119-1123). An antibody, nucleic-acid binding protein, receptor ligand, and a nucleic acid are specifically [very] efficient, and combining with each one of affiliated "joint partners" under suitable conditions is known. This phenomenon is frequently used for recognition and a diagnosis of a pathogen (for example, HIV), pathological conditions (for example, cancer, liver disease, kidney disease, a denaturation articular disease, etc.), drug abuse (for example, detection of a product called the cotinine etc.), etc.
[0005]
Many illness markers and pathogen markers (for example, protein and/or a nucleic acid) are common knowledge, and have characterized completely. Therefore, the joint partners (for example, a nucleic acid, an antibody, etc.) who combine with such a marker specifically are compounded and/or isolated, and it can be used as a marker for recognition of an illness condition or a pathogen (Landegren242:229 [Science] (1988), Mikkelson(1996) Electroanalysis, 8:15-19). Various assays are daily performed in the microbiology laboratory or the pathology laboratory using such an approach.
[0006]
Generally in a molecule nucleic-acid HAIBUDAIZESHON, an antibody ligation reaction, a protein ligation reaction, and a lectin ligation reaction [whether it inserts (to for example, double helix of DNA) and] Or are detected by use of the indicator which is one of whether it is fixed to either a target or a probe molecule by covalent bond. (For example) SosnowskiProc(s)(1997).Natl.Acad.Sci.USA, 94:1119-1123, LePecq and Paoletti(1966) Anal.Biochem., 17:100-107, Kapuscinski And Skoczylas(1977) Anal.Biochem., 83:252 -257 reference. It is used in order that electrochemical luminescence may also

detect the electrical activity luminescence marker inserted depending on the case (Pollard-KnightAnal(s)(1990).Biochem., 185:84-89, Pollard-KnightAnal(s)(1990).Biochem., 185:353-358, TizardProc(s)(1990).Natl.Acad.Sci.USA, 12:4514-4518). All of these detection strategies are either in front of the ligation reaction between a probe and a target molecule, or the back, and need derivatization of a target or a probe molecule (to for example, insertion or indirect indicator attachment sake). (to for example, covalent-labeling attachment sake) This brings about a contamination problem. Furthermore, when two or more analyte is analyzed by coincidence, two or more indicators must be used. Furthermore, complicated sample handling is required, and it increases the risk of contamination further, and it leads to/or the mistaken analysis. The above and other problems are conquered by this invention.

[0007]

(Epitome of invention)

This invention offers the new device and new approach of detecting and/or quantifying two or more analyte in a sample. This invention offers the flow through minute fluid (for example, capillary tube) biosensor which detects the target analyte (for example, nucleic acid) from which it differs in a sample, after combining with each one of affiliated "joint partners" (for example, a nucleic acid, an antibody, lectin, etc.). Generally, the section into which capillary tube channels differ [a joint partner "a probe" specific to various analyte] for example, using a photosensitive biotin / avidin technique is fixed. The flash plate of the sample is carried out into a capillary tube after that, consequently the target analyte combines with the joint partner (trapping agent) fixed by the capillary tube wall, and the remaining samples are eluted from a capillary tube. Finally, the analyte (it joined together) in which complex was formed is emitted along with the overall length of a channel, it passes a detector and a flash plate is carried out. In a desirable operation gestalt, the target-analyte which carried out desorption is detected using a sinusoid voltammetry in the copper electrode arranged down-stream (Singhal and Kuhr(1997) Anal.Chem., 69:3552-3557, SinghalAnal(s)(1997).Chem., 69:1662-1668). The time amount from elution of the target analyte to detection is used in order to determine the true character of each analyte. It is the molecule of the same kind (for example, wholly nucleic acid), or two or more analyte of a different kind (for example, protein and a nucleic acid) does in this way, and can diagnose using a single biosensor. The sensor is specific to altitude by a specific joint partner's use, and high sensitivity very much by electrochemical detection.

[0008]

Therefore, in 1 operation gestalt, this invention offers the device which detects two or more analyte in a sample. This device is set here including the channel to which each joint partner of two or more analyte is being fixed. When each joint partner of two or more analyte is stationed to the field to which channels differ, the channel has the cross-sectional area small enough and the inside of a channel is emitted to the analyte by it from two or more joint partners at flowing fluid The analyte is spatially separated until it reaches the detection point which met the down-stream channel from the joint partner or its edge, and the detector which detects the analyte on the detection point.

[0009]

Channels may be all expedient channels, such as a capillary tube, capillary-electrophoresis tubing, a channel etched into the front face, and a channel formed with the non-dense liquor printed on the front face. A channel can essentially have all dimensions, as long as it fully continues dissociating so that it may be identified, when the analyte arrives at the detection field or channel edge in a channel. A desirable channel has the cross section which gives less than about one Reynolds number (Re). a desirable channel -- about 500 micrometers or less -- more -- desirable -- about 100 micrometers or less -- it has the cross-section diameter or width of face of about 50 micrometers or less most preferably. an especially desirable device -- setting -- two or more target analyte -- at least 3 -- desirable -- at least 4 -- at least 5 and the analyte (and joint partner from whom the large number so differ) from which at least 10, at least 50, at least 100, or at least 500 differs most preferably are included more preferably. Although not restricted to below, a variety of joint partners including an antibody, binding protein, and a nucleic acid are suitable. Similarly, many detectors are suitable and a spectrophotometer (for example, absorbance spectrophotometer) and (all amperometries, a voltammetry, the potential difference, and/or a

coulometric-analysis detector are essentially included) a electroanalysis-detector are mentioned. A voltameter, especially a sinusoid voltameter are mentioned as a desirable detector.
[0010]
In another operation gestalt, this invention offers the approach of detecting two or more target analyte in a sample. This approach the fluid containing the process;ii sample which offers the detection device indicated in this specification A channel is passed under the conditions which the target analyte which exists in a fluid combines with each one of joint partners, respectively. The process which detects the analyte in the location which met the down-stream channel from the process;iv joint partner who emits the analyte to the flow of the fluid passed along with the process;iii channel which codes the analyte spatially along with a channel by that cause from a joint partner is included. Indicator attachment of the analyte is not carried out in a desirable approach. In a desirable operation gestalt, indicator attachment of the analyte is not carried out especially. an especially desirable device -- setting -- two or more target analyte -- at least 3 -- desirable -- at least 4 -- at least 5 and the analyte from which at least 10, at least 50, at least 100, or at least 500 differs most preferably are included more preferably (and so, the joint partner from whom the large number differ exists in the channel containing a detection device). Induction of the fluid flow is carried out by differential pressure and/or the electroendosmose style in some desirable operation gestalten. Fluid flow. As a "sample" fluid desirable for detection of the analyte, blood, plasma, a blood serum, urine, the liquid in the oral cavity, cerebrospinal fluid, and lymph are mentioned. Detection can be based on various approaches including a spectrophotometer (for example, absorbance spectrophotometric analysis) and (all amperometries, a voltammetry, the potential difference, and/or coulometric analysis are essentially included) the electroanalysis-approach. The desirable detection approaches are a voltammetry, especially a sinusoid voltammetry. Especially, in a desirable approach, the analyte is a nucleic acid and detection detects the target analyte by the concentration below 1x10-9M.
[0011]
(Definition)
In this specification, the vocabulary "a polypeptide", a "peptide", and "protein" are used possible [transposition], in order to point out the polymer of amino acid residue. These vocabulary is applied not only to the amino acid polymer which is the artificial chemical analog of the amino acid which exists in the nature to which one or more amino acid residue corresponds but the amino acid polymer which exists naturally.
[0012]
As the vocabulary "an antibody" is used in this specification An intact immunoglobulin, A Fv fragment only including the variable region of a light chain and a heavy chain, the Fv fragment combined by the disulfide bond (BrinkmannProc(s)(1993).Natl.Acad.Sci.USA, 90:547-551), Fab or (Fab) '2 fragmentation containing the parts of a variable region and a constant region, The antibody containing a single strand antibody etc. by which various gestalten were embellished or changed is included (Huston et al. [BirdScience(s)(1988) 242:424- 426;] (1988) Proc.Nat.Acad.Sci.USA 85:5879 -5883). An antibody may be an animal (especially a mouse or a rat) or the Homo sapiens origin, or may be a chimera (Morrison et al. (1984) Proc Nat.Acad.Sci.USA 81:6851 -6855) or hominization (JonesNature (s)(1986) 321:522-525; and open British Patent application #8707252).
[0013]
The member of the vocabulary "a joint partner", a "trapping agent", or a "joint pair" says other molecules and the molecule combined specifically, in order to form junctional complexes, such as an antibody-antigen, a lectin-carbohydrate, a nucleic-acid-nucleic acid, and biotin-avidin. In a desirable operation gestalt, association is mainly especially materialized by the noncovalent bond (for example, ion, canal) interaction.
[0014]
when pointing out biomolecules (for example, protein, a nucleic acid, an antibody, etc.) so that it may be used by this detail letter, the ligation reaction which determines existence of the biomolecule in the different-species ensemble of a molecule (for example, protein and other biologicalses) is said

[vocabulary / "it joins together specifically"]. Therefore, specific ligand or a specific antibody is combined with the specific "target" molecule under the specified conditions (for example, the immunoassay conditions in the case of an antibody or the stringent hybridization conditions in the case of a nucleic acid), and it does not join together in other molecules which exist in a sample, and a significant amount.

[0015]

The vocabulary "a channel" says the path which draws the flow of a fluid in the specific direction. A channel can be formed as the slot which has a pars basilaris ossis occipitalis and a flank, a trench, or "tubing" surrounded completely. With a part of operation gestalten, a channel does not have even the need of having a "flank." For example, a hydrophobic polymer can be applied to a flat front face, and the flow of the fluid in the front face can be restricted and/or guided in the narrow (for example, hydrophilic property) range by it. A channel is preferably equipped with at least one front face where joint partner (capture) drugs may be fixed.

[0016]

The "target analyte" is all the units or two or more molecules which should be detected and/or quantified in a sample. As desirable target analyte, biomolecules, such as a nucleic acid, an antibody, protein, and a saccharide, are mentioned.

[0017]

The vocabulary "a micro channel" is used about the channel which has the dimension which enables low Reynolds number actuation (Re<= 1, preferably Re<= 0.1, more preferably Re<= 0.01, most preferably Re<= 0.001) in this specification. Generally low Reynolds number actuation and hydrodynamics are governed by viscous force rather than inertial force.

[0018]

A vocabulary capillary tube (capillary tube) says tubing (for example, generally the flow of low Re is given) of a narrow dimension. Generally an open end capillary tube sucks up water by capillary action, when water is contacted. Although a capillary tube is not restricted to below, it can be manufactured with many ingredients containing glass, plastics, a quartz, a ceramic, and various silicates.

[0019]

"Capillary-electrophoresis tubing" says [in / therefore / a capillary-electrophoresis device] a design and/or the "capillary tube" which is generally used, or was meant so that it might be used.

[0020]

The vocabulary "a nucleic acid", an "oligonucleotide", or at least two nucleotides by which the equivalent phrase was combined with one by covalent bond in this specification are said grammatically. Although the nucleic acid of this invention is a single strand or a double strand preferably and generally includes a phosphodiester bond, so that it may outline below depending on the case For example Phospho RUAMIDO (BeaucageTetrahedron(s) (1993)) 49 (10):1925 and bibliography;Letsinger(1970) J.Org.Chem.35:3800; -- SprinzlEur(s)(1977).J.Biochem.81:579;LetsingerNucl(s)(1986).Acids Res.14:3487; -- Sawai et al. (1984) -- Chem.Lett.805 and LetsingerJ(1988).Am.Chem.Soc.110:4470; -- and PauwelsChemicaScripta(s) (1986) 26:141 9 Phosphorothioate (MagNucleic(s)(1991) Acids Res.19:1437; and U.S. Pat. No. 5,644,048), Phosphorodithioate (Briu et al. (1989) J.Am.Chem.Soc.111 : 2321), O-methyl phosphoroamidite (O-methylphophoroamidi te) association () [Eckstein, Oligonucleotides and Analogues:A Practical Approach,] [Oxford] University Refer to Press, And a peptide nucleic-acid frame And association () [Egholm] (1992) J.Am.Chem.Soc.114:1895; -- Meier et al. (1992) -- Chem.Int.Ed.Engl.31:1008;Nielsen(1993) Nature -- 365:566;CarlssonNature(s) (1996) The nucleic-acid analog including 380:207 reference which may have a mutual frame is contained. Other similar nucleic acids An electropositive frame (Denpcy et al. (1995) Proc.Natl.Acad.Sci.USA 92:6097), a nonionic frame (U.S. Pat. No. 5,386,023 and 5,637,684 --) 5,602,240, 5,216,141 And 4,469,863;Angew. Chem.Intl.Ed.English (1991) 30:423;Letsinger(s) (1988) J .Am.Chem.Soc.110:4470;Letsinger(s) (1994) Nucleoside & Nucleotide 13:1597; -- Chapter 2 -- and ASC Chapter 3 Symposium Series 580, "Carbohydrate Modifications in Antisense Research", Y.S.Sanghui, and P.Dan The volume on Cook; [Mesmaeker et al. (1994),] Bioorganic & Medicinal

Chem.Lett.4:395;JeffsJ(1994).Biomolecular NMR 34:17;Tetrahedron Lett.37:743 (1996), And U.S. Pat. No. 5,235,033, 5,034,506, the ibid of Chapter 6, and Chapter 7 () [ASC Symposium Series 580,] ["Carbohydrate Modifications] in Antisense Research", Y.S.Sanghui, and P.Dan A thing equipped with the non ribose frame which contains the thing of a publication in the volume on Cook is included. The nucleic acid containing one or more carbocyclic saccharides is also contained in the inside of a definition of a nucleic acid (refer to Jenkins et al. (1995), and Chem.Soc.Rev.169 -176 pages). Some nucleic-acid analogs are indicated by Rawls (Rawls, C&E News Jun.2, 1997 or 35 pages). These qualification of a ribose-phosphate frame can be performed in order to make addition of an additional part called an indicator easy or to increase the stability and the half-life of the molecule concerned in a physiological environment.
the vocabulary -- "-- ** -- it hybridizes specifically -- " -- and "specific hybridization" -- and -- "-- ** -- it hybridizes alternatively -- " -- alternative association of a nucleic-acid molecule to a specific nucleotide sequence, doubleness, or high buri die JINGU is said under stringent conditions as used by this detail letter. A probe hybridizes the vocabulary "stringent conditions" on the target sequence and selection target, and whether it being made extent with few other arrays and the conditions which fitted in again and which are not a comb are said. the stringent hybridization and the stringent hybridization washing conditions in a situation of nucleic-acid hybridization -- an array -- it is anaclitic and differs under a different environmental parameter. Comprehensive guidance of nucleic-acid hybridization For example, Tijssen(1993) Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes Section I, Chapter 2, and Overview of principles of hybridization and the strategy of nucleic acid probe It is found out by assays, Elsevier, and N.Y. (Tijssen). Generally, highly, by regular ionic strength and pH, stringent hybridization and stringent washing conditions are chosen so that lower about 5 degrees C than the thermal melting point (Tm) of a specific array. Tm(s) are the probe with which 50% of the target sequence agreed completely, and temperature to hybridize (under regular ionic strength and pH). Very stringent conditions are chosen so that equally to Tm about a specific probe. An example of the stringent hybridization conditions for the hybridization of a complementary nucleic acid which has the complementary residue which exceeds 100 in an array or a filter in Southern blotting or a Northern blot A standard hybridization solution It uses and is 42 degrees C (for example). [one to three Sambrook(1989) Molecular Cloning:A Laboratory Manual(s) (the 2nd edition), Cold Spring Harbor Laboratory,] [Cold] Spring Harbor Reference and hybridization are performed in all night in Press, NY, and the following detailed explanation. An example of stringent washing conditions is 0.15M [for about 15 minutes and 72 degrees C] highly. It is NaCl. In the case of the item of the SSC buffer solution, an example of stringent washing conditions is 0.2xSSC washing at 65 degrees C for 15 minutes (for example, refer to above-shown Sambrook). Mostly, in order to remove a background probe signal, low stringency washing precedes with high stringency washing. For example, an example of stringency washing of whenever [for doubleness of the nucleotide exceeding 100 / middle] is 45-degree C 1xSSC for 15 minutes. For example, examples of low stringency washing for doubleness of the nucleotide exceeding 100 are 40 degrees C 4x - 6xSSC for 15 minutes.
[0021]
A difference of localization of concentration distribution of the molecule (for example, analyte) of two or more kinds [in / in "spatial separation" / a fluid stream] is said. When the analyte is separated spatially (that is, flow coding was carried out), even if the type of all the signals of the analyte is the same, it will be possible to detect the signal according to individual of each target analyte. Therefore, the location or time amount along "passage" of detection can determine the true character of the analyte, and the difference of an indicator related to each analyte is not required.
[0022]
The electroanalysis-approach says the approach of using a system or the "electric" properties (for example, resistance, conductance, capacitance, an impedance, etc.) of the analyte, in order to take out the information about the system. As the electroanalysis-approach, all amperometries, a voltammetry, the potential difference, and/or the coulometric-analysis approach are essentially mentioned. As the desirable electroanalysis-approach, cyclic voltammetry, an alternating current, a direct current or a

rotation ring disk voltammetry, a sinusoid voltammetry, impedance spectroscopy, etc. are mentioned.
[0023]
The vocabulary "cyclic voltammetry" or an "aging voltammetry" is used possible [transposition], in order to point out cyclic voltammetry. The vocabulary "a sinusoid voltammetry" is used in order to point out cyclic voltammetry generally (for example, based on one containing a square wave, a triangular wave, etc. of aging electrical potential differences although not restricted to below), or in order to point out the use of a large amplitude sine wave potential wave used for U.S. Pat. No. 5,650,061 in a mode similar to cyclic voltammetry as a publication.
[0024]
(Detailed explanation)
(I. The efficient detection approach of two or more analyte)
This invention offers the new approach and new machine for quick detection of two or more analyte in a sample, and/or quantification. In 1 desirable operation gestalt, this invention contains the channel which fixed the specific joint partner in it in the analyte expected detection. Since a different joint partner is stationed to the field to which channels differ, when the analyte is combined, they are coded by each one of locations which met the channel at "space target." The combined analyte is behind released from a joint partner, or the inside of a channel is emitted to a joint partner / analyte complex into flowing fluid from the wall of a channel. As [separate / spatially / the analyte / until the analyte reaches the detection point of a down-stream channel from the above-mentioned joint partner / as for the dimension of a channel / continue]
[0025]
If the analyte or the analyte / joint partner complex is emitted to flow, they will be coded spatially. That is, it depends for each one of locations to both streams on the location of a joint partner when they are being fixed to the channel wall. Therefore, the time difference between emission and detection can be used in order to identify specifically the specific (or it does not generate) analyte which generates an output signal.
[0026]
Since the analyte may be identified specifically, without using an indicator in order to distinguish them from other analyte respectively, a large number, redundant sample handling, and a labeling process are eliminated. This removes many labeling and contamination problems. Moreover, the risk of the sample contamination which may lead to an incidence rate with high false positivity is also reduced or eliminated.
[0027]
It is mentioned especially that it is exchangeable to the inside and outside of the device with which it can prepare good and various minute fluid structures (for example, channel) perform flow of sample handling and a fluid and analyte detection before a channel's using it. It can have according to the analyte of the set from which a different channel differs, and the same or two or more different channels may be performed by coincidence.
[0028]
Therefore, the approach and device of this invention fit detection of the analyte in a clinical environment good. The capacity to detect unguided object-ized analyte (for example, DNA, mRNA, etc.) simplifies a procedure remarkably, and supports sample contamination and the mistaken prevention of the problem of discernment.
[0029]
Use of the copper electrode according [on 1 especially desirable operation gestalt and] to a cyclic (for example, sinusoid) voltammetry conquers many of problems which the conventional electrochemical measuring method encounters, and enables detection of the analyte by it. The high sensitivity of the detection strategy originates in the effective decoupling of the faraday signal from the capacitive background current in a frequency domain. It can follow, for example, ssDNA and dsDNA can be detected in a picomole concentration range, and an electrochemical signal originates in oxidation of the saccharide which can be accessed easily [the periphery of a DNA double helix] compared with ssDNA

of the same size.
[0030]
The sensor which can detect two or more targets only using one detector offers a cheaper and small detection system also with easy manufacture.
[0031]
(II. system component)
(A) Channel
(1) The type and dimension of a channel
A channel is suitable for operation of this invention also by what type of channel as a matter of fact, as long as passage of the matter inside a channel is enabled without being accompanied by essential mixing between the components in a solution in a different location which met the channel. That is, it is spatially continued by separating [the "down-stream" detection point] the analyte (or reagent in which other detection is possible) emitted first in the location according to individual which met the channel from the initial emission point in a desirable capillary tube. Even if, even if the type of the signal about all of analyte is the same, the capacity for the signal according to individual of each target analyte to be detectable is called spatial separation. Therefore, the time amount of the location along "passage" or detection can determine the true character of the analyte, and the difference of an indicator related to each analyte is not required.
[0032]
However, spatial separation does not require the perfect separation between analyte. Considerable overlap can be existed on the contrary, peak concentration can be detected, and a related concentration profile is measured and/or calculated and can give a positivity / electronegative detection, and/or perfect analyte quantification.
[0033]
A channel especially desirable to use by this invention is a "micro channel." The vocabulary "a micro channel" is used about the channel which has the dimension which enables low Reynolds-number actuation, i.e., the thing by which the dynamics of a fluid is governed by viscous force rather than inertial force, in this specification. The Reynolds number called ratio of inertial force to viscous force by the way is given by the following.
[0034]
Re=rho d2-/eta tau+rho ud/eta
As for a velocity vector and rho, fluid density and eta of u are time scales from which, as for the viscosity of a fluid, and d, the property dimension of a channel changes, and, as for tau, a rate changes here (being here u/tau=delta u/dt). The vocabulary "a property dimension" is here and is used as everyone knows about the dimension which determines the Reynolds number by this work. In the case of a cylindrical shape channel, it is a diameter. In the case of a rectangle channel, it is fundamentally dependent on the smaller one of width of face and the depth. It means saying that it is dependent on the width of face of the crowning of "V" in the case of V typeface channel. Count of Re about various morphological channels can be seen in the standard textbook of hydrodynamics (for example, Granger (1995) Fluid Mechanics, Dover, N.Y.;Meyer(1982) introduction to Mathematical Fluid Dynamics, Dover, N.Y.).
[0035]
The behavior of the flow of the fluid in a steady state (tau->infinity) is characterized by Reynolds number Re=rho ud/eta. The hydraulic system by which micro processing was carried out is in a low Reynolds-number regime (Re is less than about one) mostly for small size and a low speed. In this regime, a turbulent flow and a secondary flow, therefore the inertia effectiveness of flowing and producing mixing inside can be disregarded, and viscous effectiveness governs dynamics. Generally under such conditions, the flow in a channel is stratified.
[0036]
Since a Reynolds number is dependent not only on a channel dimension but the time scale from which fluid density, fluid viscosity, a fluid rate, and a rate change, the absolute upper limit of a channel

diameter is not specified clearly. According to the channel geometrical configuration actually designed good, the high processing ability system which can avoid about R< 1000 if it depends especially about R< 100, therefore has large channel size relatively is possible for a turbulent flow. Desirable channel property dimension range is about 0.5 micrometers thru/or 100mm. Especially a channel range with a property dimension of about 1 micrometer - about 100 micrometers is desirable, and about 5 micrometers - about 100 micrometers are the most desirable. More desirable range is about 5 micrometers thru/or 50 micrometers.

[0037]

The device of this invention does not need to be restricted to low Reynolds number actuation. a signal with different analyte mutual when a joint probe is estranged widely and the analyte so emitted is widely estranged in flow -- "overlap ****" -- remarkable convective mixing may occur in a channel, without carrying out a mask. Furthermore, as long as remarkable mixing of two analyte may occur and remarkable (for example, it is statistically significant) space separation exists between the peak concentration of two analyte, he can distinguish a signal and it will be understood that detection of each analyte can carry out. However, quantification of the analyte according to each may become gradually more difficult as the analyte mixes each other. Nevertheless, even such a situation can obtain quantification by evaluating or modeling the spatial distribution of the analyte based on the location and the rate of fall-off of a concentration peak, in order to give approximation of an integral signal to each analyte.

[0038]

As long as above-mentioned mixed requirements are fulfilled as above-mentioned, all channel configurations are proper. Therefore, although not limited to a suitable channel below, the channel formed of an obstruction [which counters], open slot, and closed ditch etc. is included. As for a channel, the shape of the shape of circular, a rectangle, a rectangle, a triangle, and v character and u character, a hexagon, an octagon, an irregular form, etc. can have all cross sections as a matter of fact. The channel used in this invention does not need to be continuous. It can follow, for example, a channel can be formed by the aggregate, a copolymer, or cross linked polymer of a porous particle etc.

[0039]

As long as the ingredient is essentially stable to the solution which passes through the inside of it, all channel ingredients fit operation of this invention. or [that a desirable ingredient is combinable with a joint partner] -- or as it joins together, can derivatize or it is a joint partner's linker. Furthermore, in a desirable operation gestalt, an ingredient is chosen and/or reformed so that it may not combine with the analyte substantially. Moreover, it does not combine with a probe in the field besides the reason expected to fix a probe, or a desirable ingredient does not interact to another appearance.

[0040]

Although especially a desirable ingredient is not limited to below, it contains glass, silicon, a quartz or other minerals, plastics, the ceramics, a metal, paper, a metalloid, a semi-conductor, cement, etc. Furthermore, the matter which forms gels, such as protein (for example, gelatin), a lipopolysaccharide, a silicate, agarose, and polyacrylamide, can be used. A variety of organic polymers and inorganic polymers of nature and both composition may be used as an ingredient on the front face of a solid-state. An instantiation-polymer contains polyethylene, polypropylene, Pori (4-methylbutene), polystyrene, polymethacrylate, Pori (ethylene terephthalate), rayon, nylon, Pori (vinyl butyrate), poly vinylidene JIFURUORIDO (PVDF), silicon, polyformaldehyde, a cellulose, cellulose acetate, a nitrocellulose, etc.

[0041]

In the case of conductivity or a semi-conductive substrate, an insulating layer exists in a substrate preferably. This is especially important when a device incorporates an electro-technical element (for example, the direction system of an electric fluid, a sensor, etc. move an ingredient around a system using the electroendosmose force). the application for which, as for a substrate ingredient, they are meant in the case of a polymer substrate -- responding -- hard, half rigidity or non-hard one, and opacity -- suppose that it is translucent or transparent. For example, it is manufactured by the transparent material optically partially [in order that the device containing a visual-detection element may enable

the detection or may generally support it at least] at least. Or glass or the transparent aperture of a quartz may be taken in by the device about the detecting element of such a format again. Additionally, a polymer ingredient has a straight chain or a branching principal chain, and a bridge is constructed over it or it can presuppose un-constructing a bridge to it. Especially the example of a desirable polymer ingredient contains for example, poly dimethylsiloxane (PDMS), polyurethane, a polyvinyl chloride (VPC), polystyrene, polysulfone, a polycarbonate, etc.

[0042]

A channel can be used as the component of a larger body. Therefore, a channel can be assembled with other one or more channels, in order to obtain many channels, and assay from which plurality differs by it can be performed to coincidence. A channel can be used as the component of a machine including suitable liquid handling, detection and/or sample handling / application function.

[0043]

moreover, a channel can carry out "plug-in" to the machine which performs assay of this invention suitably -- it can manufacture as a unit of reusable or throwing away. Although a channel is not limited to below, it is understood that it can prepare or more for any one in a variety of bodies containing a micro titration pan (for example, PVC, polypropylene, or polystyrene), a test tube (glass or plastics), dip sticks (for example, glass, PVC, polypropylene, polystyrene, a latex, etc.), a micro centrifuge tube or glass, a silica, plastics, a metal, or a polymer bead.

[0044]

With a desirable operation gestalt, one or more channels are especially manufactured as an element of the "integrated circuit" which is prepared in glass or a silicon slide as a capillary tube channel, or has an onboard circuit element for control of liquid flow, application of a sample, and/or detection of a signal as capillary tube tubing (for example, capillary-electrophoresis tubing). In the most desirable operation gestalt, as illustrated in the example in this specification, it has a channel as capillary tubes, such as capillary-electrophoresis tubing.

[0045]

(2) Channel manufacture

The approach of manufacturing the channel of this invention is well-known to this contractor. For example, when a channel is formed from one or more capillary tubes, a capillary tube is purchased from a commercial contractor (for example, Polymicron Technologies, Tucson, Az), or by the conventional capillary tube "drawing" **, it can draw out or extrude and it can be carried out.

[0046]

When manufacturing a channel on a front face, they can be formed by standard technique, for example, machining, shaping, sculpture, etching, a laminating, extrusion, or deposition is possible for them.

[0047]

In 1 desirable operation gestalt, a channel is manufactured using a well-known micro-machining process (for example, photolithography) in solid-state electronic industry. Usually, a micro device, for example, a micro channel, is created in the form of the semiconductor wafer used in order to manufacture an integrated circuit from a semi-conductor substrate called extensively available crystal silicon or glass. Manufacture of the micro device from a semiconductor wafer substrate can utilize a broad experience of both the surface etching technique developed by the semi-conductor processing industry for integrated-circuit (IC) manufacture, and bulk etching technique for the similarity of an ingredient.

[0048]

In order to create a movable element, surface etching used in order to form a thin surface pattern in a semiconductor wafer in IC manufacture is correctable so that sacrifice undercut etching of the thin layer of a semiconductor material may be enabled. Bulk etching used in case a deep trench is generally formed in a wafer using an anisotropic etching process in IC manufacture can be used in order to machine an edge or a trench to a precision in a micro device. In order to remove the ingredient by which a mask is not carried out from a wafer, "wet processing" which uses chemicals called a pottasium hydroxide solution can perform both surface etching and bulk etching of a wafer. In order to form various channel elements in micro device creation, it is even possible to use the anisotropy wet

processing technique which depends on the distinctive crystal orientation of an ingredient, or is dependent on use of an electrochemical dirty stop.

[0049]

Generally another etching processing technique which allows the considerable freedom of a micro device design is known as "dry etching processing." Especially this processing technique is suitable for the anisotropic etching of the fine structure. Dry etching processing contains many gaseous phases or plasma phase etching technique which attains to even a little isotropic low energy plasma technique which guides the plasma stream which contains chemical reactivity ion in order to carry out induction of the formation of an volatile resultant to a wafer from the high anisotropy sputtering process which carries out the impact of the wafer with a high energy atom or ion in order to move a wafer atom to a gaseous phase (for example, ion beam milling).

[0050]

There is an especially useful dry etching process known as reactive ion etching in the middle of high energy sputtering technique and low energy plasma technique. Reactive ion etching is accompanied by guiding an ion content plasma stream to a semi-conductor or other wafers for instantaneous sputtering and plasma etching. Reactive ion etching holds some of profits of an anisotropy related to sputtering, though reactant plasma ion is offered for formation of the gaseous-phase-reaction product which answered contact of reactant plasma ion with a wafer. The rate of wafer ingredient removal is actually remarkably reinforced to either the sputtering technique performed independently or low energy plasma technique. Therefore, reactive ion etching has possibility of becoming the etching process which excelled for micro device creation by the ability of a high anisotropy etching rate being maintained relatively. An above-mentioned micro-machining technique is well-known to this contractor like many other things (for example, refer to Choudhury(1997) The Handbook of Microlithography, Micromachining, and Microfabrication, Soc.Photo-Optical Instru.Engineer, and Bard & Faulkner(1997) Fundamentals of Microfabrication). Furthermore, the example of use of the micro-machining technique in silicon or a borosilicate glass chip can be seen to U.S. Pat. No. 5,194,133, 5,132,012, 4,908,112, and 4,891,120.

[0051]

In 1 operation gestalt, in a silicon (100) wafer, in order to carry out pattern formation of a channel and the connection, a standard photolithography technique is used for a channel and micro processing is carried out. In order that ethylenediamine and a pyrocatechol (EDP) may be used for two-step etching and may give a closed liquid system, anode plate junction of the Pyrex (trademark) (Pyrex) 7740 cover plate can be carried out in the field of silicon. In this case, liquid connection can be made behind silicon.

[0052]

In a desirable operation gestalt, a channel can be manufactured from other capillary tubes, such as glass, a quartz, or capillary-electrophoresis tubing, as above-mentioned.

[0053]

With other operation gestalten, in order that a channel may form a channel wall, by making a substrate deposit an ingredient, it can manufacture (using sputtering or other joining techniques), or casting/shaping of a channel may be done in an ingredient. Although casting / shaping channel is not restricted to below, it is easily manufactured from a variety of ingredients containing various metals, plastics, or glass. In a specific desirable operation gestalt a channel Various elastomers for example, alkylation chlorosulfonated polyethylene (Acsium (trademark)) -- A polyolefine elastomer (for example, Engage (trademark)), Chlorosulfonated polyethylene (for example, Hypalon (trademark)), A perfluoro-elastomer (for example, Kalrez (trademark)), neoprene polychloroprene, Casting is carried out with an ethylene-propylene-diene terpolymer (EPDM), chlorinated polyethylene (for example, Tyrin (trademark)), and various siloxane polymers (for example, poly dimethylsiloxane etc.).

[0054]

(B) Joint partner

one or more pieces by which the channel used by this invention was fixed to one or more front faces in

the desirable operation gestalt -- biological -- a "joint partner" is held. biological -- the constituent of a "joint partner" or a "joint pair" says other molecules, the molecule combined specifically, or a presentation, in order to form junctional complexes, such as an antibody-antigen, a lectin-carbohydrate, a nucleic-acid-nucleic acid, and biotin-avidin.
[0055]
when pointing out biomolecules (for example, protein, a nucleic acid, an antibody, etc.) so that it may be used by this detail letter, the ligation reaction which determines existence of the biomolecule different-species ensemble of protein and other biologicalses is said [vocabulary / "it joins together specifically"]. Therefore, appointed ligand or an appointed antibody is combined with the specific "target" (for example, protein or a nucleic acid) under the specified conditions (for example, the immunoassay conditions in the case of an antibody or the stringent hybridization conditions in the case of a nucleic acid), and it does not join together in other molecules and a significant amount.
[0056]
The joint partner used in this invention is chosen based on the target identified / quantified. It follows, for example, when a target is a nucleic acid, a joint partner is a nucleic acid or nucleic-acid binding protein preferably. When a target is protein, a joint partner is the receptor, the ligand, or the antibody preferably combined with the protein specifically. When a target is a saccharide or a glycoprotein, a joint partner is lectin etc. preferably.
[0057]
Although a proper joint partner (trapping agent) does not restrict to below, he contains a nucleic acid, protein, receptor binding protein, nucleic-acid binding protein, lectin, a saccharide, a glycoprotein, an antibody, a lipid, etc. Such a joint partner's composition or isolation approach is well-known to this contractor.
[0058]
(1) Preparation of a joint partner (trapping agent)
(a) Nucleic acid
The nucleic acid for using it as a joint partner in this invention can be manufactured or isolated according to either of the approaches of well-known a large number to this contractor. With 1 operation gestalt, a nucleic acid can consider as the isolated spontaneous generation nucleic acid (for example, genomic DNA, cDNA, mRNA, etc.). The method of isolating a spontaneous generation nucleic acid is well-known to this contractor (for example, refer to SambrookMolecular(s)(1989) Cloning-A Laboratory Manual (2nd edition), one to three-volume, Cold Spring Harbor Laboratory, Cold Spring Harbor, and N.Y.).
[0059]
However, in a desirable operation gestalt, a nucleic acid is created newly (de novo) by chemosynthesis. With a desirable operation gestalt, a nucleic acid (for example, oligonucleotide) An automatic composition device is used for Needham-VanDevanter and others (Needham-VanDevanterNucleic(s) (1984) Acids Res., 12:6159-6168) as a publication. Beaucage and Caruthers (it Caruthers(es) (1981) Beaucage and [ ] --) Tetrahedron According to the solid phase phospho lamination DAITO triester method which Letts. and 22 (20):1859-1862 indicated, it is compounded chemically. When required, generally purification of an oligonucleotide is performed by Pearson and Regnier (Pearson and Regnier (1983) J.Chrom.255:137-149) by either native acrylamide gel electrophoresis or the anion exchange HPLC as a publication. The array of an synthetic oligonucleotide can be checked using the chemical degradation method of Maxam and Gilbert (Maxam and Gilbert(1980) in Grossman and Moldave(piece) Academic Press, New York, Meth.Enzymol.65:499-560).
[0060]
(b) An antibody/antibody fragment
The antibody or antibody fragment for using it as a joint partner (trapping agent) It can manufacture by the approach of well-known many to this contractor. (For example) Harlow & Lane(1988) Antibodies:A Laboratory Manual, Cold Spring Harbor Laboratory, and Asai(1993) Methods in Cell Biology The 37th volume: Antibodies in Cell Biology, Academic Refer to Press and Inc.N.Y. It sets to one approach and

an antibody is produced by making an animal (for example, rabbit) into immunity by the immunogen containing an epitope [hoping to recognize/capture]. It can be used in order that much immunogens may produce a specific reaction nature antibody. Recombination protein is immunogen desirable to production of a monoclonal antibody or a polyclonal antibody. Moreover, the protein which exists naturally can also be used by one of the pure or impure gestalten. A synthetic peptide is similarly created by standard peptide synthetic chemistry. for example, Barany and Merrifield, and Solid-Phase Peptide Synthesis; -- 3 - 284 pages The Peptides:Analysis, Synthesis, and Biology. -- the 2nd -- volume:Special Methods in Peptide Synthesis and Part A. -- [Merrifield] (1963) J.Am.Chem.Soc., 85:2149-2156, and Stewart(s) (1984) Solid Phase Peptide Synthesis, the 2nd edition, Pierce Refer to Chem.Co., Rockford, and Ill.

[0061]

The production approach of a polyclonal antibody is common knowledge at this contractor. If it says simply, the immunogen which is the cytoskeleton component refined preferably will be mixed with an adjuvant, and an animal will be made into immunity. Trial bleeding is performed for the immunoreaction to the immunogen pharmaceutical preparation of the animal, and it supervises by determining the reactant potency to a cytoskeleton component and a test presentation. When a high potency is obtained appropriately [the antibody to immunogen], blood is collected from an animal and antiserum is prepared. In a necessary case, in order to condense about a reactant antibody for a cytoskeleton component, the further fraction of antiserum can be performed. . (see the above-shown Harlow & Lane).

[0062]

This contractor can get a monoclonal antibody by the various techniques of concordance. If it says simply, generally immortalization of the spleen cell from the animal made into immunity with the desired antigen will be carried out by fusion to a myeloma cell (Kohler and Milstein(1976) Eur.J.Immunol.6:511 -519 reference). The alternate method of immortalization includes the transformation by other well-known approaches by the Epstein-Barr virus, the oncogene, the retrovirus, or this business. The yield of the monoclonal antibody which screens about an antigen the colony produced from a single immortalization cell for production of desired singularity and the antibody of an affinity, and is produced by such cell can be reinforced by various techniques including the impregnation to a vertebrate host's peritoneal cavity. Or it is also possible to isolate the DNA sequence which carries out the code of a monoclonal antibody or its joint fragmentation by screening a DNA library from a Homo sapiens B cell again according to the general protocol outlined by Huse and others (246:1275-Huse1281 [Science] (1989)).

[0063]

For example, production/selection also of antibody fragments, such as a single strand antibody (scFv or in addition to this), can be done using a phage display technique. The capacity which can discover an antibody fragment on the front face of the virus (a bacteriophage or phage) with which bacteria are infected makes it possible to isolate a single joint antibody fragment from the library of the uncombined clone exceeding 1010. In order to make an antibody fragment discover on the surface of phage (phage display), it is inserted in the gene to which an antibody fragment gene carries out the code of the phage surface protein (pIII), and antibody fragment-pIII fusion protein is displayed by the phage front face (Hoogenboom et al. [McCafferty / Nature / 348:552- 554;] (1990) (1991) Nucleic Acids Res. 19:4133 - 4137).

[0064]

Since the antibody fragment on the front face of phage is functionality, the phage holding an antigen joint antibody fragment can be isolated from uncombined phage with antigen affinity chromatography (348:552-McCafferty554 [Nature] (1990)). Depending on the affinity of an antibody fragment, a 1,000,000 times [20 times to] as many enrichment factor as this is obtained about one affinity sorting. However, more phage can be proliferated and one sorting can be made to already be received by infecting bacteria with the eluted phage. Thus, the 1000 times as many concentration by 1 time as this can increase 1,000,000 times in two sortings (348:552-McCafferty554 [Nature] (1990)). time

[therefore,] concentration is low -- (-- MarksJ(1991).Mol.Biol.222:581-597) and affinity sorting of multiple times may bring about isolation of rare phage. Since sorting of the phage antibody library of an antigen produces concentration as a result, a large majority of clones combine an antigen after about three - four sortings. Therefore, it is necessary to analyze a small number of (hundreds) clone only about association with an antigen comparatively.

[0065]

By displaying various very large and V gene repertories on phage, a Homo sapiens antibody is not based on the conventional immunization, but can be produced (Marks et al. (1991) J.Mol.Biol. 222:581 -597). In 1 operation gestalt, natural VH and natural VL repertory which exist in a human peripheral blood lymphocyte were isolated from the non-immunizing donor by PCR. The splice of both the V gene repertories was carried out at random using PCR, the scFv gene repertory was produced, the clone of this was carried out to the phage vector, and it produced the library of a 30 million phage antibody (this writing). The joint antibody fragment was isolated from this single "unsettled" phage antibody library to a different antigen exceeding 17 containing hapten, polysaccharide, and protein (Clackson et al. [Marks et al. / MarksJ(1991).Mol.Biol.222:581- 597; / (1993) .Bio/Technology. 10:779-783;GriffithsEMBO(s) (1993) J.12:725- 734;] (1991) Nature. 352:624 -628). The antibody was produced to the self-protein containing the thyroglobulin, an immunoglobulin, a human tumor necrosis factor, and human CEA (Griffiths et al. (1993) EMBO J.12:725 -734). Moreover, it is also possible by sorting out directly in a cell as it is to isolate the antibody to a cell surface antigen. The antibody fragment is very specific about the antigen used for sorting, and it has the affinity of the range of 1:M-100nM (Griffiths et al. [MarksJ (1991).Mol.Biol.222:581- 597;] (1993) EMBO J.12:725 -734). A bigger phage antibody library produces isolation of the antibody of twist a large number of the high joint affinity to the antigen of a larger rate as a result.

[0066]

(c) Binding protein

In 1 operation gestalt, a joint partner (trapping agent) may be binding protein. Although proper binding protein is not restricted to below, it contains a receptor (for example, cell surface receptor), receptor ligand, cytokine, a transcription factor and other nucleic-acid binding protein, a growth factor, etc.

[0067]

Protein can make mutation able to induce from the protein which isolated from the source of nature or was isolated, or can be compounded newly. A means to isolate the protein which exists naturally is well-known to this contractor. Although such an approach is not restricted to below, ammonium-sulfate precipitate, an affinity column, The well-known protein purification approach containing a column chromatography, gel electrophoresis, etc. is included (generally). R. Scopes and Protein (1982) Purification, Springer-Verlag, N.Y.;Deutscher(1990) Methods in The 182nd volume of Enzymology: Guide to Protein Purification and Academic Refer to Press and Inc.N.Y.

[0068]

When protein combines a target reversibly, the affinity column holding a target may be used in order to carry out affinity purification of the protein. Or it can rearrange with a HIS tag, and a target can be discovered, and protein can also be refined using a nickel2+/NTA chromatography.

[0069]

With another operation gestalt, protein may be chemically compounded using a standard chemical peptide synthesis technique. When a desired array is comparatively short, a molecule may be compounded as a single continuous polypeptide. When asking for a larger molecule, a partial array is compounded separately (in one or more units), and can be united by forming peptide linkage after that by the condensation of the amino terminus of one molecule, and the carboxyl terminus of the molecule of another side. This is performed using the same chemistry (for example, Fmoc, Tboc) as being typically, used in order to combine single amino acid in the peptide synthesis machine for commerce.

[0070]

After the C-terminal amino acid of an array is fixed to insoluble support, the solid phase composition accompanied by sequential addition of the remaining amino acid of an array is the approach that it is

desirable for the chemosynthesis of the polypeptide of this invention. About the technique of solid phase composition Barany And Merrifield () [Barany] and Merrifield(1962) Solid-Phase Peptide Synthesis;3-284 page and The Peptides:Analysis and Synthesis -- Biology. 2nd volume:Special Methods in Peptide Synthesis, Part A., Merrifield et al. (MerrifieldJ(1963).Am.Chem.Soc., 85:2149-2156) -- and It is indicated by Stewart and others (StewartSolid(s)(1984) Phase Peptide Synthesis, the 2nd edition, Pierce Chem.Co., Rockford, Ill).

[0071]

In a desirable operation gestalt, it may be compounded using recombinant DNA methodology. Generally, this includes the process which reproduces protein, when still more nearly required, the process which creates the DNA sequence which carries out the code of the binding protein, the process which puts DNA on the manifestation cassette under a specific promotor's control, the process which makes protein discover in a host, the process which isolates the discovered protein, and.

[0072]

DNA which carries out the code of the binding protein or the array of this invention For example, cloning of a suitable array and a limit Or Narang's and others phospho triester method (Narang et al. (1979) Meth.Enzymol. 68:90 -99), Brown's and others phosphodiester method (Brown et al. (1979) Meth.Enzymol. 68:109 -151), Beaucage's and others diethyl phospho lamination DAITO -- law (BeaucageTetra(s)(1981).Lett., 22:1859-1862) -- and It can prepare by the suitable approach including the direct chemosynthesis by the approach of the solid support method of U.S. Pat. No. 4,458,066 of the above arbitration.

[0073]

The nucleic-acid array which carries out the code of the desired binding protein may be discovered in the various host cells containing various high order eukaryotic cells, such as Escherichia coli, other bacteria hosts, yeast, COS and CHO, a HeLa-cell stock, and a myeloma cell system. A recombination protein gene may be connected possible [suitable about each host / an expression control array and actuation]. In the case of Escherichia coli, this contains the conclusion signal of an imprint as preferably as promotors, such as T7, trp, or lambda promotor, and a ribosome bond part. In the case of an eukaryotic cell, a regulatory sequence may include a splice donor and a receptor array preferably, including a promotor, the enhancer guided from an immunoglobulin gene, SV40, a cytomegalovirus, etc., and a polyadenylation array.

[0074]

A plasmid may be imported into the selected host cell by the well-known approach of the calcium phosphate processing or electric punching of a case of the calcium chloride transformation in the case of Escherichia coli, and a mammalian cell. The cell in which a transformation is carried out by the plasmid can be chosen by the resistance to the antibody given with the gene contained in plasmids, such as an amp gene, a gpt gene, a neo gene, and a hyg gene.

[0075]

Once it is discovered, recombination binding protein may be refined according to the protocol of the criterion of these above business.

[0076]

(d) A saccharide and a carbohydrate

A saccharide and a carbohydrate are included as other joint partners. A saccharide and a carbohydrate can be isolated from the source of nature, can be compounded with an enzyme, or can be compounded on a chemistry target. The path for production of specific oligosaccharide class formation is in. It is based on use of the enzyme (glycosyltransferase) which produces them by vivo. such an enzyme -- in of oligosaccharide REJIO for vitro composition -- it may be used as alternative and a stereo alternative catalyst (Ichikawa et al. (1992) Anal.Biochem. 202:215 -238). A sialyltransferase may be used in combination with an auxiliary glycosyltransferase. For example, the combination of a sialyltransferase and galactosyltransferase can be used. In order to compound desired oligosaccharide class formation, many approaches of using a glycosyltransferase are well-known. The instantiation-approach is indicated by WO 96/32491, Ito et al. (Ito et al. (1993) Pure Appl.Chem. 65:753), U.S. Pat. No. 5,352,670, No.

5,374,541, and No. 5,545,553. By being combined in an early reaction mixture, instead, an enzyme and a substrate can add the reagent of an enzyme and the 2nd glycosyltransferase circuit to a reaction medium, when the first glycosyltransferase circuit approaches completion. By carrying out two glycosyltransferase circuits in order in a single container, the whole yield improves rather than the protocol with which a middle kind is isolated.

[0077]

The approach of chemosynthesis is indicated by Zhang (ZhangJ(1999).Am.Chem.Soc., 121(4):734-753). If it says simply, the set of the base unit of the sugar base will be created by this approach by each base unit preloaded in a different protective group. A base unit is graded by the reactivity of each protective group. After that, it is correctly determined which fundamental component must be added to a reaction so that the reaction of a single string [computer program] from the fastest thing to the thing of the maximum ** may produce a desired compound.

[0078]

(2) Adhesion of the joint partner to a channel

Many approaches of fixing biomolecule on various solid-state front faces are well-known in the field concerned. Covalent bond of the desired component is carried out, or it may be fixed by the noncovalent bond through specific or nonspecific association.

[0079]

When covalent bond is desired between a compound and a front face, a front face is polyfunctional or can usually carry out [many organic functions]-izing. The functional group which may be used for association may contain a carboxylic acid, an aldehyde, the amino group, a cyano group, ethylene, hydroxyl, a sulfhydryl group, etc. by existing in a front face. The approach of connecting various compounds with various front faces is common knowledge, and is illustrated by reference at abundance. For example, Ichiro Please refer to Chibata (Ichiro Chibata(1978) Immobilized Enzymes, Halsted Press, New York) and Cuatrecasas (Cuatrecasas, J(1970).Biol.Chem.245:3059).

[0080]

In addition to covalent bond, the various approaches of combining an assay component by the noncovalent bond can be used. Generally a noncovalent bond is nonspecific adsorption of the compound to a front face. Usually, a front face is blocked with the 2nd compound in order to prevent nonspecific association of an assay component by which the indicator was carried out. Or although it combines a front face with one component nonspecific, another thing is designed so that it may hardly join together. For example, with a carbohydrate content compound, the front face which holds lectin called concanavalin A will not be combined with the protein lacking in glycosylation by which the indicator was carried out, although it joins together. The various solid-state front faces for using it for immobilization by the noncovalent bond of an assay component are outlined in U.S. Pat. No. 4,447,576 and 4,254,082.

[0081]

A molecule is in when a joint partner is a nucleic acid or a polypeptide. It is chemically compoundable by situ. this permutes an optical instability protective group by the usual protective group (for example, dimethoxytrityl radical used in nucleic acid biosynthesis (DMT)) -- the standard chemosynthesis approach is included substantially. The exposure of the micro channel in the dispersed location produces alternative association with a monomer (for example, amino acid or a nucleotide), and the growth polypeptide of the irradiated part or a nucleic acid as a result. The approach of optical directivity polymer composition is common knowledge at this contractor (for example, U.S. Pat. No. 5,143,854, the PCT public presentation number WO 90/15070, WO 92/10092 and WO 93/09668, further 251,767 to 77 refer to [Science] the Fodor (1991)).

[0082]

In a desirable operation gestalt, a joint partner may be fixed using a linker (for example, gay 2 functionality or a hetero bifunctional linker). A linker proper in order to combine a biological joint partner is common knowledge at this contractor. For example, although protein or a nucleic-acid molecule is not restricted to below, it may be combined by either of the various linkers containing a

peptide linker, a straight chain, or a branched-chain chain linker, or the heterocycle type carbon linker. A hetero bifunctional crosslinking reagent called the activity ester of N-ethyl malei mide it is used widely (Lerner et al. [for example,] (LernerProc(s)(1981).Nat.Acad.Sci.USA, 78:3403-3407) --) Kitagawa et al. (KitagawaJ(1976).Biochem., 79:233-236), Refer to Birch and Lennox (Birch and Lennox (1995) of Chapter 4, Monoclonal Antibodies:Principles and Applications, Wiley-Liss, N.Y.).

[0083]

With 1 desirable operation gestalt, a joint partner is fixed using a biotin / avidin interaction. In this operation gestalt, the biotin or avidin which has an optical instability protective group may be arranged at a channel. The exposure of the channel of the location according to individual produces association of the biotin to the channel in the location, or avidin. Then, the binder holding each biotin or avidin is arranged at a channel, and it combines with each joint partner and is localized in an exposure part. It may be repeated in the location according to each where this process is wanted to fix a joint partner.

[0084]

Sigrist and others (SigristBio/Technology (1992), 10:1026-1028) has indicated the another suitable photochemistry-joint approach. By this approach, the interaction of ligand with an organic front face or an inorganic front face is mediated with the polymer which has the carbene which generates trifluoromethyl-aryl-JIAJIRIN which works as a linker molecule and which can be photoactivated. Photoactivation of the aryl-JIAJIRINO functional group in 350nm produces a highly reactive carbene, and covalent bond is attained by coincidence carbene insertion on both ligand and an inactive front face. Therefore, a reactant functional group is not required in which of ligand or the charge of supporting material.

[0085]

In the most desirable operation gestalt, in order to cover a fused silica front face with an organic coating, the thin layer of an epoxy resin (Epotek350) is applied to a fused silica (fused cilica) capillary tube (bore of 50 micrometers). A surface organic coating not only makes the minimum DNA adsorption in a capillary tube wall, but offers the polymerization-ized front face which can carry out direct immobilization of the DNA probe. Liu and others (Liu et al. (1996) J.Chromatogr. 723:157 -167) has indicated the protocol which covers a capillary tube front face with an epoxy resin. It dried under the nitrogen pressure of 20psi, and when saying simply, after carrying out the rinse of the capillary tube for 15 minutes with an acetone first, it was dried in oven at 100 degrees C for 1 hour. Epoxy resin 314ND (Epo-Tek, Billerica, MA) was dynamically covered on the capillary tube front face by attracting the acetone solution of epoxy resin mixture. The residual solvent was removed from the epoxy resin covering capillary tube by pouring nitrogen for 30 minutes at a room temperature. The bridge was constructed at 150 degrees C in 30 minutes and under the nitrogen pressure of after that 20psi by 80 degrees C in epoxy resin coating for 2 hours. The buffer solution washes the applied capillary tube for 30 minutes before use.

[0086]

Then, a specific DNA probe solution is poured into 1cm section of an epoxy resin covering capillary tube. In order that a DNA probe solution may combine a DNA probe with a capillary tube wall by the hydrophobic interaction and the electrostatic interaction, it is made to react with the piece of a capillary tube overnight. Other DNA probes are fixed like the piece of a covering capillary tube of same 1cm length. Once it is fixed by the capillary tube wall, after those hybridization fields' being deionized water and rinsing, the preparation assembled to the capillary tube biosensor which has a joint partner who is different in a different location will be completed.

[0087]

(C) The analyte detection approach

All approaches can be used according to the approach of this invention on the fact of biological molecule detection. Since the true character of various analyte is determined by those spatial positions in the flow which is moving the inside of a channel, there is no need for a different labeling system about each analyte. I hear that one advantage of this assay system, not to mention it, does not have the need of attaching an indicator in the analyte, and it is in it.

[0088]
The method of detecting the analyte is common knowledge at this contractor. When the analyte is labeled (for example, radioactivity, fluorescence, the MAG, or a mass indicator), the analyte is detected by detecting an indicator. However, with a desirable operation gestalt, the analyte is not labeled and it does not depend for the desirable detection approach on use of the indicator attached to the analyte. Although such a detection approach is not restricted to below, it includes detection of detection (for example, radiation and/or an absorption spectroscopy) of a lightwave signal, the electrical and electric equipment, and a magnetic signal, and detection of change of the electrical characteristics (for example, conductance/resistance, capacitance, an impedance, etc.) of the medium containing the analyte.
[0089]
With 1 simple operation gestalt, optical absorption of the fluid containing the analyte is supervised by the detector (for example, standard ultraviolet rays). However, a electroanalysis-detector is used with a desirable operation gestalt. With the most desirable operation gestalt, a electroanalysis-detector uses a voltammetry (for example, sinusoid).
[0090]
In a desirable operation gestalt, a sinusoid voltammetry includes supplying the analyte of the little purpose to a voltammetry electrode especially. A sine (or others carry out aging) electrical potential difference is applied to an electrode. The electrical potential difference which carries out aging (for example, sine) is the single period of a predetermined frequency, and in order to carry out the sweep of the formal potential of the target redox kind, it has the amplitude large enough. The response of the analyte to a sinusoidal voltage is alternatively detected by the higher harmonic of the fundamental frequency of an aging electrical potential difference. The method of performing an aging voltammetry is offered in the bibliography quoted U.S. Pat. No. 5,650,061 and in it.
[0091]
Although especially a desirable operation gestalt uses a sinusoid voltammetry, other voltammetry approaches are suitable for this invention good. As above-mentioned, especially the aging voltammetry approach is desirable, and such a voltammetry approach is not restricted to the use of an electrical potential difference in which a sine wave carries out aging. Moreover, other waves are suitable. Although such an approach is not restricted to below, it includes use of a square wave and a triangular wave (triangle wave). Such an aging voltammetry approach is common knowledge at this contractor (7 (1): for example, Cullison and Kuhr (1996) Electroanalysis, 1-6 reference).
[0092]
Discovery of this invention was that combination with the analyte isolation by which the code was carried out to sinusoid voltammetry detection and a space target provides altitude with detection/quantum of the specific analyte on very low level in a complicated sample (for example, blood serum).
[0093]
(III. integrated assay device)
The newest chemical-analysis system used in chemical manufacture, environmental analysis, a medicine diagnosis, and fundamental experiment room analysis has the capacity of full-automatic-izing preferably. Such a comprehensive analysis system (TAS) () [Fillipini] (1991) J.Biotechnol.18:153;Garn (s) (1989) Biotechnol .Bioeng.34:423;Tshulena(1988) Phys.Scr.T23:293;Edmonds(1985) Trends Anal.Chem.4:220 --;StinshoffAnal(s)(1985).Chem.57:114 R;Guibault(1983) Anal.Chem Symp.Ser.17:637;Widmer(1983) Trends Anal.Chem.2:8 perform automatically the function which attains to detection including transportation of the sample in a system, sample preparation, isolation, purification, data collection, and evaluation from installation of the sample to a system.
[0094]
In recent years, the sample preparation technique is reduced with the sufficient result to the miniaturized gestalt. Therefore, for example, a gas chromatography (Widmer et al. (1984) Int.J.Environ.Anal.Chem. 18:1), High pressure liquid chromatography (MullerJ(1991).High) Resolut.Chromatogr.14: 174;Manz (es) (1990) Sensors & Actuators Microcolumn edited (1985) by B1:249;Novotny Separations:Columns,

Instrumentation and Ancillary Techniques J. Chromatogr.Library, Micro-Column edited (1984) by 30th volume;Kucera High Performance Liquid The volume Chromatography, Elsevier, and on Amsterdam;Scott (1984) Small Bore Liquid Chromatography Columns:Their Properties and Uses, Wiley, and N.Y.;JorgensonJ(1983). -- Chromatogr.255:335;KnoxJ(1979).Chromatogr.186:405; -- Tsuda et al. (1978) -- Anal.Chem.50:632 -- and capillary electrophoresis (ManzJ(1992).Chromatogr.593:253; -- Olefirowicz et al. (1990) -- Anal.Chem.62:1872;Second Int'l Symp.High-Perf.Capillary) Electrophoresis(1990) J.Chromatogr.516; -- Ghowsi et al. (1990) -- Anal.Chem.62:2714 are reduced with the sufficient result to the miniaturized gestalt.

[0095]

Similarly, it sets in another operation gestalt and this invention offers the integrated assay device (for example, TAS) which detects and/or quantifies many analyte. An assay device contains a channel equipped with the joint partner fixed as above-mentioned. Furthermore, a desirable integrated assay device is one or more reservoirs which supply :detection system (for example, voltammetry system containing an electrode and/or related electronics), the buffer solution, and/or the Flushing fluid containing following one or more, a sample application well and/or an inlet, and a computer controller (control, such as a pump, a reservoir flow change, a detector, and a signal analysis system, sake).

[0096]

With a desirable operation gestalt, an integrated assay device equips a "removable" unit with a channel especially. When the capillary tube which follows, for example, can be easily inserted, and detached and attached from an accompanying device may be prepared in a module as a channel, thereby, a device can work easily by the assay of the set with which analyte differs.

[0097]

When the channel used with a device is tubing (for example, capillary-electrophoresis tubing), the conventional capillary-electrophoresis device is equipped with the subordinate piping and sample handling component, feeding component, and computer controller for the "integrated" assay device according to many this inventions. In order to offer the integrated assay device which fits detection and/or the quantum of various analyte good, it is hardly the need other than quite simple installation/addition of the detector (for example, sinusoid voltammetry detector) according to this invention, and related electronics.

[0098]

(Activation of IV. assay)

Generally, assay is performed by introducing a sample into the channel which has the fixed joint partner. A sample is kept suitable for the bottom of the condition each joint partner enables it to combine with the target analyte which may exist in a sample specifically. Then, the flash plate of the sample is carried out by installation of the buffer solution which supports emission of the analyte generally combined from a channel. The analyte emitted after that is detected by the down-stream detection point, and the true character of the analyte is determined by the time amount from emission to detection.

[0099]

(A) Preparation of a sample

as a matter of fact -- oh, a ** sample can analyze using this advantageous device and advantageous approach. However, with a desirable operation gestalt, a sample is a biological sample. The vocabulary "a biological sample" says the sample obtained from the component (for example, cell) of an organism or an organism so that it may be used in this specification. A sample can be taken as the thing of all biological organizations or a fluid. In many cases, a sample is a "clinical sample" which is a sample originating in a patient. Although such a sample is not restricted to below, it contains expectoration, cerebrospinal fluid, blood, a blood fraction (for example, a blood serum, plasma), a corpuscle (for example, leucocyte), an organization or a fine needle biopsy sample, urine, ascites and pleural effusion, or the cell originating in them. Moreover, a biological sample may also contain an organization intercept called the frozen section obtained for the purpose on histology.

[0100]

** of a biological sample (for example, blood serum) by which the direct method of analysis is carried

out is also good, and before use by the assay of this invention, a certain preparation may be presented with them. Although such preparation is not restricted to below, it may include removal of the cell residual dust by suspension / dilution, or centrifugal separation of a sample in water or the suitable buffer solution etc., or selection of the specific fraction of the sample before analysis.
[0101]
(B) Feeding of the sample to a system
A sample can be introduced into the device of this invention according to the standard method of common knowledge to this contractor. By following, although a sample is used in a high-pressure liquid chroma TOGURAFISHI stem, it can be introduced into a channel through an inlet [like]. With another operation gestalt, a sample is applicable to the sample well connecting with a channel. In still more nearly another operation gestalt, pump feeding of the sample may be carried out into a channel. The approach of introducing a sample into a channel is common knowledge, and a criterion in the technique of capillary electrophoresis and a chromatography.
[0102]
(C) Connection condition
A sample will be maintained at the basis of the conditions which promote specific association between a sample and a joint partner once it goes into a channel. The conditions which suit specific association between a joint partner and the analyte are common knowledge at this contractor. For example, the buffer solution suitable in order to promote association between an antibody and target protein is common knowledge in an immunoassay technique. for example, U.S. Pat. No. 4,366,241 -- No. 4,376,110 No. 4,517,288 and No. 4,837,168; Asai(1993) Methods in Cell Biology Volume 37:Antibodies in Cell Biology and Academic Press, Inc.New York;Stites & Terr(1991) Basic and Clinical Immunology 7th Refer to Edition. Similarly, the conditions at the time of a nucleic acid hybridizing specifically mutually are also common knowledge at this contractor (refer to above-shown Tijssen (1993)). A specific connection condition is optimized by this contractor about the specific set of a joint partner and the target analyte according to a well-known standard method. for example, Above-shown Tijssen (1993) and U.S. Pat. No. 4,366,241 -- No. 4,376,110, No. 4,517,288 and No. 4,837,168; Asai(1993) Methods in Cell Biology Volume 37:Antibodies in Cell Biology and Academic Press, Inc.New York;Stites & Terr(1991) Basic and Clinical Immunology 7th Refer to Edition.
[0103]
(D) Emission conditions
They are emitted after the analyte in a sample is specifically combined with the joint partner who fixed to the channel. Emission is suitably performed by the temperature conditions which contact a joint partner / analyte complex to the buffer solution and which are caused especially or destroy the interaction of a joint partner / analyte. Such a meeting may be destroyed by use of an elevated temperature, modifiers (for example, a urea, a formamide, etc.), quantity or low pH, quantity or low-salt, and various chaotropic agents (for example, guanidine hydrochloride) according to the pair of specific analyte / joint partner.
[0104]
(E) Analyte/flow in a channel
A sample, and/or a carrier / buffer-solution fluid can be introduced to a channel according to the standard approach, and can move the inside of/or a channel. For example, a fluid may be introduced and moved into a channel by the simple gravity feed from a "reservoir." Or the inside of a channel may be moved to a fluid by the pressure to the fluid pressure, and the deformable chamber/diaphram produced with either gas pressure or the various suitable pumps (for example, a peristaltic pump, a measuring pump, etc.) etc. again. Moreover, the inside of a channel may be moved also to the analyte by the electroendosmose approach.
[0105]
(F) Detection
Analyte detection can be based on either of many approaches of common knowledge to these above contractors as above-mentioned. In the desirable operation gestalt, the electrochemical detection

approach was used and detection is based on the sinusoid voltammetry with the most desirable operation gestalt.
[0106]
The protocol for performing a sinusoid voltammetry is already indicated (Singhal et al.(1997) Anal.Chem.69:4828-4832; and U.S. Pat. No. 5,650,061). If it says simply, digital generation of the sine wave of 2Hz, 0.7 ****-p, and +0.35V direct current offset will be carried out using a software program. This sine wave is committed as impression potential to a copper electrode. The current responses from an electrode are collected by software on real time between the single overall lengths of an elution run. This time amount domain current response is changed into a frequency domain by the fast Fourier transform after that. The protocol for analyzing frequency spectrum is mentioned already (Singhal et al. (1997) Anal.Chem.69:1662-1668). The spectrum corresponding to the analyte is obtained after background subtraction and a digital phase lock as stated above (above-shown Singhal et al. (1997)).
[0107]
(V. Kit for two or more analyte detection)
In 1 operation gestalt, this invention offers the kit screened in order to identify or quantify many the existence of the analyte or the absences in a sample. A kit contains the channel of this invention holding the various joint partners fixed on the surface of each one as it is shown in this specification. a channel may be designed for the simple and quick nest to an one apparatus assay device called a device equipped with the computer control system for control of analysis of suitable piping for maintenance of management of an electrochemical detector (for example, sinusoid voltammetry) circuit and a sample, and the flow of the fluid in a channel and application of a sample, the flow of a fluid, and a signal output as explained for example, to this detail in the letter. A kit can contain further the suitable buffer solution for use, other solutions, and the standard substance in the assay approach described into this specification.
[0108]
Furthermore, a kit may contain teaching materials including the directions (namely, protocol) for enforcing the approach of this invention. Although teaching materials generally contain a document or printed matter, they are not restricted to such a thing. Such directions are stored and all the media that can transmit them to an end user are taken into consideration by this invention. Although such a medium is not restricted to below, it contains an electronic storing medium (for example, a magnetic disk, a tape, a cartridge, a chip), an optical medium (for example, CD-ROM), etc. Such a medium may include the address to the Internet site which offers such teaching materials.
[0109]
(Example)
The following examples are not for showing in order to illustrate the invention in this application, and limiting.
[0110]
(Example 1)
(Electrochemical detection of the nano liter volume of DNA hybridization)
(An ingredient and approach)
(Reagent)
After deionizing the water to be used, it passed the Milli-Q water purification system (Millipore Corp., Bedford, Mass.). About tuberculosis (TB), a biotin-ized DNA probe specific to identification of a human immunodeficiency virus (HIV), and a cDNA target, it is Genemed. Special order composition was carried out through Synthesis and Inc. (San Francisco, Calif.) (Table 1). The DNA probe solution was produced by diluting 100microg [/ml] solution of the DNA probe dissolved in deionized water into 1:1 mixture with a DNA binding solution (Pierce Chemicals, CA). This joint solution makes it easy to combine DNA with a polymerization-ized front face by the canal and the electrostatic interaction. The fused silica capillary tube (Polymicron Technologies, Inc., AZ) was used in order to produce a capillary tube biosensor. The flash plate of these capillary tubes is not carried out with an acetone, but it was made to dry before performing a certain derivatization on a capillary tube front face.

[0111]
(Capillary tube derivatization and immobilization of a DNA probe)
The fused silica capillary tube (the bore [of 50 micrometers] x outer diameter of 150 micrometers, die length of 1m) was used for the biosensor. The capillary tube was covered with the thin layer of an epoxy resin (Epotek350) in order to cover a fused silica front face with organic covering. Surface organic covering not only makes DNA adsorption of the wall of a capillary tube the minimum, but gives the polymerization-ized front face where a DNA probe may be fixed directly. The protocol which covers a capillary tube front face with an epoxy resin was as Liu and others (Liu et al.(1996) J.Chromatogr.723:157-167) having explained correctly. It dried under the nitrogen pressure of 20psi, and when saying simply, after carrying out the rinse of the capillary tube for 15 minutes with an acetone first, it was dried in oven at 100 degrees C for 1 hour. Epoxy resin 314ND (Epo-Tek, Billerica, MA) was dynamically covered on the capillary tube front face by attracting the acetone solution of epoxy resin mixture. The residual solvent was removed from the epoxy resin covering capillary tube by carrying out a flash plate with nitrogen for 30 minutes with a room temperature. Epoxy resin covering constructed the bridge over the pan at 150 degrees C under the nitrogen pressure of 20psi(s) for 30 minutes by 80 degrees C for 2 hours. The buffer solution washed the covered capillary tube for 30 minutes before use.
[0112]
Subsequently, the flash plate of the 1cm section of an epoxy resin covering capillary tube was carried out with the specific DNA probe solution. The DNA probe solution was made to react with the piece of a capillary tube overnight, and the DNA probe was combined with the capillary tube wall by the canal and the electrostatic interaction. Other DNA probes were fixed like the piece of a covering capillary tube of same 1cm length. When the probe was fixed by the capillary tube wall, after being deionized water and rinsing those hybridization fields, the preparation assembled to a capillary tube biosensor was completed. The distance from an inlet port to the 1st probe (TB probe) is about 25cm, and these hybridization fields were pasted up on the "separation column" with the epoxy resin in two different locations which the probe whose number is two left 15cm. Thereby, the distance of about 60cm was left behind from the 2nd probe (HIV probe) even to the detector. It connected by [both] pasting up a capillary tube on the sleeve (180x360-micrometer capillary tube section) which is die length of about 1cm respectively too about the segment from which a capillary tube differs with an epoxy resin. The overall length of a capillary tube biosensor was about 1m.
[0113]
(DNA label-hybridization, elution, and detection)
The capillary tube was attached in the capillary-electrophoresis device for commerce (Biorad Instruments Inc, Hercules, CA), and this device was used for that pressurization flow and an automatic-sampler function. The protocol used in order to hybridize a complementary target with high stringency to these DNA probes is extensively indicated by reference. The specific protocol used for this experiment is as follows.
[0114]
First, in order to make a probe and a selection target combine a cDNA target, the flash plate of the capillary tube was carried out with the pre hybridization buffer solution (inside of 0.75M NaCl, 75mM sodium citrate, a pH=7.0 or 0.1%N-lactoyl sarcosine, 0.02%SDS, and 50% formamide, 40 degrees C). The flash plate of the DNA target solution of both TB and a HIV target was dissolved and carried out to the pre hybridization buffer solution, it incubated for about 30 minutes in the capillary tube, and the perfect hybridization and the saturation of a surface fixed probe were obtained.
[0115]
Subsequently, it is the hybridization buffer solution (0.3M NaCl, 30mM sodium citrate, pH=7.0 or 0.1% SDS), and it was begun to rinse a surplus target solution. After that, in order that the DNA target as for whom any did nonspecific association might also remove, the stringent washing buffer solution (75mM NaCl, a 7.5mM sodium citrate, pH=7.0 or 0.1%SDS, 40 degrees C) performed stringent washing. Since all other things were probed under these stringent conditions by this stringent washing, by it, it was guaranteed that only a complementary DNA target is completely left behind to the interior of a capillary

tube biosensor.
[0116]
Subsequently, in order to begin to rinse the high stringency washing buffer solution which does not suit a capillary tube with a copper electrode (to the existence of a surface active agent sake), it filled up with the electrochemical washing buffer solution (89mM TRIS, 89mM boric acid and 1mM EDTA, pH=10).
[0117]
When the capillary tube was filled up with the electrochemical washing buffer solution, the copper electrode was once maintained at the biosensor capillary tube outlet. The electrode was automatically aligned with the capillary tube outlet by two PERT machining design (two-part machined design) (Kuhr (1993) U.S. Pat. No. 5,650,061). Subsequently, the elution buffer solution (89mM Tris, 89mM boric acid and 1mM EDTA, pH=11) was filled quickly (with 100psi), and the capillary tube was incubated for 30 minutes at the room temperature. The elution buffer solution promoted the hybridized DNA label-denaturation, and emitted oligomer into the solution inside a capillary tube by it in the specific location.
[0118]
Subsequently, pump feeding of the elution buffer solution including the dehybridized target DNA was carried out by the fixed rate of flow using the pressurization induction flow by about 5 psi(s), and the DNA target emitted when they moved with the buffer solution by it was eluted. When DNA target oligomer passes a detector and flowed, DNA oxidized by electrocatalysis by the copper electrode, and, thereby, generated the signal which may be detected using a sinusoid voltammetry as stated above (refer to U.S. Pat. No. 5,650,061). After that, the zone according to each [of DNA] was detected by the copper electrode of an outlet, when DNA passed a detector and moved.
[0119]
(Electrochemical detection)
Copper microelectrode with a diameter of 40 microns was manufactured inside 5cm and 50x360-micrometer fused silica capillary tube. The capillary tube was filled with the gallium using the syringe. Next, after inserting the copper wire of small die length in a capillary tube by the end, it sealed in the proper place by epoxy resin adhesion for 5 minutes. Another wire was inserted from the back end of a capillary tube, and electrical connection with a copper wire was given. The gallium inside a capillary tube gave the electrical connection between two wires. Such capillary tube microelectrodes are very strong, and reusable after polish. These electrodes were not pretreated with any gestalten except polish by the hand using the sandpaper of 600 grain size.
[0120]
The sinusoid voltammetry was used in order for copper microelectrode to detect the dehybridized DNA target, when it is eluted from a capillary tube. The protocol for performing a sinusoid voltammetry is mentioned already (Singhal et al.(1997) Anal.Chem.69:4828-4832; U.S. Pat. No. 5,650,061). When saying simply, digital generation of the sine wave of 2Hz, 0.7 ****-p, and +0.35V direct current offset was carried out using the software program in a company. This sine wave was committed as impression potential to a copper electrode. The current responses from an electrode were collected with software on real time between the single overall lengths of an elution run. Subsequently, this time amount domain current response was changed into the frequency domain by the fast Fourier transform. The protocol for analyzing frequency spectrum is mentioned already (Singhal et al.(1997) Anal.Chem.69:1662-1668). The spectrum corresponding to the analyte was obtained after background subtraction and a digital phase lock as stated above (above-shown Singhal et al. (1997)).
[0121]
(A result and consideration)
Since DNA is clinically important as an index of a disease, the amount of low of DNA hybridization and direct detection are desirable. Once it is shown that a specific nucleotide sequence is connected with a predetermined marker (for example, an infectious agent, an inherited character, a neoplasm type) characteristic or identifiable, the array is compounded in large quantities, and in order to determine whether the specific array exists, it can be used as a probe of a nucleic acid from other sources of supply. In many cases, the DNA assay based on hybridization is developed for the application from which many

differ, the fingerprints of the existing DNA are carried out completely, and in order to identify, two or more trials need to be performed about all samples.
[0122]
The sinusoid voltammetry which is a frequency domain voltammetry detection technique can be used in order to detect a nucleic acid under the same experiment conditions as what is used for detection of a saccharide. A nucleotide can also be contributed to a nucleobase by those bases apart from that by which a certain signal of a nucleotide is based on a sugar principal chain since they are also electrical activities on a copper front face, including an amine part.
[0123]
Detection of unguided object-ized DNA is very desirable in order to avoid all sample handling loss and a contamination problem. From what (it can work in the amount of pico liter capacity from a nano liter) can be miniaturized easily, without sacrificing the capacity as a high sensitivity detector, electrochemical detection is suitable, especially when [of DNA analysis] a sample is restricted generally.
[0124]
In development of this capillary tube biosensor, the specific array of DNA was fixed to the field to which the interior of a continuous minute fluid channel (namely, fused silica capillary tube) differs. 1cm section of 20 bore capillary tube of 50 micrometers which is in agreement with the sample volume of nL (s) was used in order to give the recognition field of a sensor. Through each field, one by one, pump feeding is carried out, and a suitable DNA target can combine a sample with a target independently with each fixed DNA probe there (if it exists). Once the sample had an opportunity to interact with the target by which each was fixed, elution of it was carried out from the capillary tube, the whole capillary tube was washed by a series of stringent washing, and all possibility of polluting an ingredient by it was eliminated. Subsequently, the target [having combined with each field of the fixed probe] DNA was eluted in the format by which the code was carried out spatially.
[0125]
Drawing 1 shows the fundamental approach used in this design, in order to give possibility of observing two or more hybridization events in a single experiment. Zones 1 and 2 are fixed zones where the DNA probe of TB and HIV was fixed, respectively. These zones were together put in order to produce a capillary tube system single in order to use one impregnation of the sample containing a DNA target behind. A reagent required in order to wash a more complicated sample (namely, clinical sample containing other biomolecules of a large number, such as protein and other cell strains) with very high stringency can be introduced by the flow by which pressurization induction was carried out from the reservoir of the head of a capillary tube. Copper microelectrode is arranged at the outlet edge of a capillary tube, and it is arranged using the machining two PERT system which enables automatic alignment of a capillary tube with an electrode (Kuhr's and others U.S. Pat. No. 5,650,061). Therefore, a system is very easy to combine, and once it works, it is dogged.
[0126]
The sequence of the process used in order to perform specific hybridization, washing, and elution of a target oligonucleotide that denaturalized is shown in drawing 2 . The same process can be used for all the DNA label kinds to those complementary probes of stringent hybridization. In this scheme,
1) Hybridization is performed under stringent conditions, in order to avoid all nonspecific label association to the probe which is not perfect phase complement to a capillary tube wall or the target analyte. Consequently, TB target (the oligomer (zone 1) which has an array characteristic of DNA which carries out the code of the TB is only hybridized to fixed TB probe (complementary sequence), and a HIV target only hybridizes it to the HIV probe (zone 2) fixed under stringent conditions.) These zones are isolated spatially and stringent washing removes all interferent components also from the capillary tube which separates those zones only from each zone.
[0127]
2) The last washing by the elution buffer solution (TBE, pH=11) denatures the hybridized complementary nucleic acid to coincidence, and emits the DNA target which joined together by it to the

solution which adjoins the fixed probe of a capillary tube directly. Such two label spatial selectivity is maintained. It is because the buffer solution moves to a proper place quickly (with time scale also with the much high-speed twist which dehybridization may produce), and the flow in a capillary tube stops and a denaturation process is completed after the incubation for 30 minutes.

[0128]

3) Finally elution of the solution containing the "free" target DNA oligomer separated spatially is carried out. Since the zone including the two targets is spatially separate, they pass over the copper electrode arranged at the outlet to different time amount, and flow to it. The scheme shown in drawing 3 has illustrated the aspect of affairs of detecting the eluted DNA target. Each label elution time amount in a detector shows the true character, and, thereby, codes the part of DNA hybridization.

[0129]

Detection of the HIV target DNA using the capillary tube biosensor by 1cm zone of a fixed DNA probe is shown in drawing 4 . The flash plate of the sample containing 10microg [/ml] 100 synthetic HIV labelmicroL was carried out through the inside of the capillary tube biosensor with which the HIV probe was fixed. In order to enable HIV oligonucleotide label-detection of a sample, it followed in order of the process indicated to drawing 2 . Originally, the sequence did not contain the electrochemical washing buffer solution (89mM TRIS, 89mM boric acid and 1mM EDTA, pH=10). It added in order to make into the minimum the artifact observed when the elution buffer solution attacks this to a copper electrode. pH of this buffer solution is important. While too high pH leads to Target's DNA dehybridization and loss of a signal is brought about, it is because too low pH produces the big artifact as a result when the elution buffer solution reaches a detector.

[0130]

As shown in drawing 4 , DNA label-elution is proved [signal / which was acquired with the sinusoid voltammetry] after the dehybridization in the elution buffer solution. Although it is shown that the elution of a blank solution has the very stable signal, it is difficult to evaluate the singularity of HIV label-association by the single probe system. Therefore, this kind of detection may bring about false positivity in a DNA trial.

[0131]

In the design of the proper, two or more probes system not only can tackle the problem of parallel processing of a nucleic-acid sample, but gives the internal reference over nonspecific hybridization. It will give two or more peaks in two or more probes system, when nonspecific hybridization occurs with a given sample. This will show the need for a much more stringent hybridization protocol directly until a peak single about the single target which poured in is detected. The singularity of the hybridization of this system is illustrated to drawing 5 (A), and detection of the specific label-hybridization of TB and HIV exists in coincidence in the same sample. Although the interaction of the sample was carried out only once to each DNA probe, two targets can detect to coincidence by one run. The transit time about two zones agrees with TB and the HIV label-internal reference which were shown in drawing 5 (B) and 5 (C), respectively. Therefore, this also shows that what kind of nonspecific hybridization which two targets not only can detect to coincidence, but occurs under the hybridization conditions currently used does not exist. Otherwise, probably, the internal reference run showed not one but two peaks (that is, even if TB specific target probably hybridized to self completely complementary probe and HIV specific probe and sticks in HIV specific label, he is the same). Therefore, detection of two peaks in drawing 5 (A) shows composite TB and HIV specific label-detection clearly to coincidence, has illustrated the absence of nonspecific hybridization, and reduces the hope of generating as a result of all false positivity.

[0132]

DNA sequencing by hybridization is dependent on the molecular recognition given by the hybridization to the fixed probe DNA of a sample (for example, target) DNA molecule. Die length is about 7 nucleotide at least, die length is about 10 nucleotide at least more preferably, die length is the nucleotide of 15 or 20 at least still more preferably, and the die length of a desirable probe oligonucleotide is the nucleotide of 30, 40, or 50 at least most preferably. This probe has a complementary known array to at

least 1 label field. Although the assay format that a large number differ exists, after a probe contacts a nitrocellulose, agarose, plastics, or a sample, is placed and washes un-recognizing [DNA] finely, it is typically fixed by other quality of a deactivating group which can carry out assay about a content. the assay of hybridized DNA is executable in the system indicated in this detail letter with the elution from denaturation, capillary tube, or channel of DNA, and detection by SV in copper microelectrode.
[0133]
(Conclusion)
The new DNA biosensor of the capillary tube base was developed using the direct electrochemical detection which can detect two or more DNA oligomers to coincidence. This detection scheme used the DNA label flow coding hybridization assay in a sample by various DNA probes fixed by the location where capillary tube front faces are various. It is supplemented with the DNA label coincidence hybridization of various types by those label-direct detection in the copper electrode by using a sinusoid voltammetry when they elute. a disease -- such detection of a specific oligonucleotide array-like in parallel and raw is dogged, and it is durable and it can open the path to a cheap two or more disease DNA sensor. therefore, it -- activation -- an operator -- the problem accompanying the existing DNA sensor based on intensive and expensive, various optical detection schemes is avoided.
[0134]
(Example 2)
(High sensitivity of the amino acid by the sinusoid voltammetry, and a peptide, and alternative detection)
(Experiment parameter)
(Reagent)
After deionizing the water to be used, it passed the Milli-Q water purification system (Millipore Corp., Bedford, Mass.). Amino acid, the insulin (98 - 99%, and Sigma Chemical Corp. and St. Louis, Mo.), and the remaining peptide (Peninsula Laboratories, Inc., San Carlos, CA) were used received. All experiments were conducted by 0.10 sodium hydroxides (A. a C.S grade, Fisher Scientific, Fair Lawn NJ) as a migration electrolyte. The undiluted solution of 0.10M was prepared in deionized water. Future dilution was performed using the migration electrolyte.
[0135]
(Copper microelectrode)
Copper microelectrode was produced by pulling a glass capillary tube by microelectrode Pullar (Model PE-2, Narishige, Tokyo Japan) first. Then, the edge of a capillary tube was cut off by Scalpel under the microscope. Then, copper wire (99.99%, Goodfellow, Cambridge, England) with a diameter of 20 micrometers was inserted in the edge from which it was cut out newly, and was sealed with the epoxy resin (Epoxy Technology, Billerica, Massachusetts). The electrode was ground by the diamond grinding wheel and carried out clarification by sonication by deionized water. In order to make electrical connection with copper wire, the back end of a capillary tube was filled up with the gallium (Sigma Chemical Co.), and diameter the copper wire of 150 micrometers was inserted in the gallium. As an alternative, the back end of a capillary tube was filled up with the epoxy resin, and more, the copper wire of a major diameter was put into the epoxy resin restoration capillary tube until it contacted 20-micrometer wire physically. Any electrochemical pretreatments are not performed, but the electrode was stabilized until the stable response of about 1 hour was observed under experiment conditions.
[0136]
(Electrochemical measurement and experiment conditions)
The flow cell was constituted from PUREKISHI glass, and tubing was adjusted so that diffusion-breadth might be avoided. Installation of a sample plug was controlled by the air operated actuator controlled by the solenoid valve. The rate of flow was maintained by the gravity flow by maintaining a buffer-solution reservoir on 19cm of a flow cell. It determined that the rate of flow was a part for 0.5ml/, and the volume of a sample was determined from the rate of flow and die length of impregnation. Impregnation time amount determined that an electrode will look at the perfect concentration of the analyte.
[0137]

The reported conditions of an experiment are explained here. In the case of amino acid and a peptide, 2Hz sine wave (0-690mV pair Ag/AgCl) applied with the software written by the author by Labview (National Instruments, Austin, Tex.). Wave filtration of the wave was carried out with the 4 super-low region filtration filter using cyberamp (Model 380, Axon Instruments Inc., Foster City, CA.) with 3db point of being 3 times (6Hz) many as fundamental frequency. Wave filtration of the output current was carried out with the 4 super-low region passage filter. The filter was set as 40Hz (4 times, the 10th higher harmonic, or 20Hz of the observed maximum frequency). A current is 300MHz. Pentium (trademark) It changed into the analog from digital ones by the 16-bit analog-to-digital converter (PCI-4451, National Instruments) using II personal computer. The single scan consisted of 4 sine-wave periods.

[0138]

With Labview software (National Instruments), the collected time amount domain was changed into the frequency domain, and was further processed using Matlab programming (The Mathworks, Inc., Englewood Cliffs NJ). The spectrum of only a signal was obtained by lengthening the background vector acquired before impregnation from an instant signal current vector. In order to acquire a time amount domain spectrum, the digital lock in amplifying method was used. In order to generate the amplitude of each frequency higher harmonic (up to the 10th higher harmonic of max), and a phase angle, the Fourier transform of the time amount spectrum was carried out at the rate of 512 points. The vector of only a signal was used for the topology of each higher harmonic wave, and it acquired it by projecting it on a background subtraction signal vector. Finally, moving-average smoothing (cube type integral) was used for the phase decomposition vector, and it carried out low-pass wave filtration passage.

[0139]

(Result)

Drawing 6 shows the background subtraction frequency spectrum of the arginine in copper microelectrode. The experiment was performed using 1microM arginine. The excitation signal was the sine wave of 2Hz and 0-690mV pair Ag/AgCl. The current from 4 sine wave periods which consist of 512 points (whole time amount = 1 second) was used in order to generate each frequency spectrum. The three-dimensions graph consists of the frequency (x axis), the amplitude (z-axis), and phase angle (y-axis) information to the 10th higher harmonic.

[0140]

Drawing 7 shows the sinusoidal time amount domain response from 1microM arginine in the 5th higher harmonic (10Hz). This higher harmonic gave the highest signal / noise ratio, and the limit of detection (S/N=3) of 39nM(s).

[0141]

Drawing 8 shows the linearity dynamic range of various arginine concentration. The arginine concentration of 1, 10,100, and 1000microM was poured into the flow impregnation analysis system. The amplitude of the 5th higher harmonic (10Hz) is plotted to four poured-in different concentration. This plot shows the outstanding linearity (R= 0.9997) covering 3 order in the 5th higher harmonic.

[0142]

Drawing 9 shows the asparagine in copper microelectrode, and the subtraction frequency spectrum of a glutamine. A square expresses 10microM asparagine and the circle expresses 10microM glutamine. Experiment conditions are the same as what was used in order to generate drawing 1 .

[0143]

Drawing 10 A and 10B show the sinusoidal time amount domain response of the asparagine in the 6th higher harmonic (12Hz), and a glutamine. Drawing 10 A shows 10microM asparagine, and drawing 10 B shows 10microM glutamine. The 6th higher harmonic has the optimization phase angle of those two amino acid closest to 90-degree separation. This higher harmonic gives the maximum selectivity in between those two analyte. In the case of an asparagine, the limit of detection (S/N=3) in this higher harmonic is 400nM(s), and, in the case of a glutamine, is 500nM.

[0144]

Drawing 11 shows the background subtraction frequency domain spectrum of 10microM insulin B chain. The same conditions as drawing 1 were used.
[0145]
Drawing 12 shows the sinusoidal time amount domain component of the insulin B chain in the 4th higher harmonic (8Hz). The 4th higher harmonic gave the greatest signal / noise ratio, and the limit of detection (S/N=3) of 500nM(s).
[0146]
Drawing 13 shows the luteinizing hormone releasing hormone (circle) in copper microelectrode, and the subtraction frequency spectrum of bradykinin (square).
[0147]
Drawing 14 A and 14B show the time amount domain response of the bradykinin in the 2nd higher harmonic wave (4Hz), and luteinizing hormone releasing hormone, respectively.
[0148]
Drawing 15 shows the background subtraction frequency domain response of neurotensin (square) and substance P (circle), respectively.
[0149]
Drawing 16 A and 16B show the time amount domain response of the neurotensin in fundamental frequency (2Hz), and substance P, respectively.
[0150]
The example and operation gestalt which were explained here are for for the purpose of instantiation, in the light of it, various corrections or modification are submitted to this contractor, and it is understood that it should be contained in the inside of the pneuma of this application, the text, and an attachment claim. Therefore, all the publications quoted here, a patent, and patent application are taken in by reference in a perfect form by all the purpose here.
[Brief Description of the Drawings]
[Drawing 1]
Drawing 1 shows the schematic drawing of the DNA biosensor of the capillary tube base by electrochemical detection. Two different probe sections exist in a capillary tube. They are the probe 1 of TB specific probe, and the probe 2 of a HIV specific probe. A HPCE automatic sampler is used for various stringent washing and rinses required for the cDNA label specific hybridization of these fixed probes. A copper electrode is arranged at the outlet of a capillary tube biosensor using a machining two PERT system.
[Drawing 2]
Drawing 2 shows the protocol for performing DNA label stringent hybridization and alkali denaturation inside a capillary tube biosensor. (1) Hybridize various DNA targets to the probe fixed by the capillary tube front face. (2) After that, stringent washing is performed in order to remove one of nonspecific adsorption or DNA which were hybridized. (3) Alkali denaturation is performed by ** which finally elutes the DNA target which hybridized from the capillary tube biosensor before.
[Drawing 3]
Drawing 3 shows the elution from a DNA label capillary tube biosensor by which alkali denaturation was carried out, and the continuing schematic drawing of electrochemical detection. An electrode is manufactured inside the piece of a capillary tube equipped with the same diameter as a biosensor capillary tube, in order to make automatic alignment easy. An electrode will carry out a location extremely at the outlet of a biosensor capillary tube soon (<5micrometer). Lower trace shows the schematic drawing of the DNA label-detection at the time of their eluting from a biosensor capillary tube.
[Drawing 4]
Drawing 4 illustrates the HIV specific label-detection which used a capillary tube biosensor and sinusoid voltammetry detection. A 10microg [/ml] HIV specific target is passed inside the capillary tube biosensor with which only the HIV specific probe was fixed. All hybridization conditions are as a publication in this specification. The sinusoid voltammetry excitation wave was 2Hz in 0 - 700 mVp-p.

The illustrated signal was acquired by the 5th higher harmonic.
[Drawing 5]
Drawing 5 shows two or more DNA label-detection which used flow coding hybridization assay for coincidence. The used sample contained 1:1 mixture of concentration with a specific label-each [of HIV and TB] of 10microg [/ml]. All hybridization and elution conditions are the same as what was explained in the thing and example 1 in drawing 4 . Since detection understood the illustrated signal that it has the best sensibility, it was acquired by the 5th higher harmonic.
[Drawing 6]
Drawing 6 shows the background subtraction frequency spectrum of the arginine in copper microelectrode. The three-dimensions graph consists of the frequency (x axis), the amplitude (z-axis), and phase angle (y-axis) information to the 10th higher harmonic.
[Drawing 7]
Drawing 7 shows the sinusoidal time amount domain response from 1microM arginine in the 5th higher harmonic (10Hz).
[Drawing 8]
Drawing 8 shows the linearity dynamic range of various arginine concentration.
[Drawing 9]
Drawing 9 shows the asparagine in copper microelectrode, and the subtraction frequency spectrum of a glutamine. A square expresses 10microM asparagine and the circle expresses 10microM glutamine.
[Drawing 10]
Drawing 10 A and 10B show the sinusoidal time amount domain response of the asparagine in the 6th higher harmonic (12Hz), and a glutamine. Drawing 10 A shows 10microM asparagine, and drawing 10 B shows 10microM glutamine.
[Drawing 11]
Drawing 11 shows the background subtraction frequency domain spectrum of 10microM insulin B chain.
[Drawing 12]
Drawing 12 shows the sinusoidal time amount domain component of the insulin B chain in the 4th higher harmonic (8Hz).
[Drawing 13]
Drawing 13 shows the luteinizing hormone releasing hormone (circle) in copper microelectrode, and the subtraction frequency spectrum of bradykinin (square).
[Drawing 14]
Drawing 14 A and 14B show the time amount domain response of the bradykinin in the 2nd higher harmonic wave (4Hz), and luteinizing hormone releasing hormone, respectively.
[Drawing 15]
Drawing 15 shows the background subtraction frequency domain response of neurotensin (square) and substance P (circle), respectively.
[Drawing 16]
Drawing 16 A and 16B show the time amount domain response of the neurotensin in fundamental frequency (2Hz), and substance P, respectively.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

TECHNICAL FIELD

(Field of invention)
This invention relates to the field of a diagnosis. This invention provides a detail with the device and approach which do not need use of an indicator or an indicator attachment process, though quick detection and/or the quick quantum of two or more analyte are made possible.
[0004]
(Background of invention)
Immunoassay and nucleic-acid hybridization chemistry perform the illness diagnosis which detects a genetic defect, and are quickly developed towards the target to perform prognostic evaluation (SosnowskiProc(s)(1997).Natl.Acad.Sci.USA, 94:1119-1123). An antibody, nucleic-acid binding protein, receptor ligand, and a nucleic acid are specifically [very] efficient, and combining with each one of affiliated "joint partners" under suitable conditions is known. This phenomenon is frequently used for recognition and a diagnosis of a pathogen (for example, HIV), pathological conditions (for example, cancer, liver disease, kidney disease, a denaturation articular disease, etc.), drug abuse (for example, detection of a product called the cotinine etc.), etc.
[0005]
Many illness markers and pathogen markers (for example, protein and/or a nucleic acid) are common knowledge, and have characterized completely. Therefore, the joint partners (for example, a nucleic acid, an antibody, etc.) who combine with such a marker specifically are compounded and/or isolated, and it can be used as a marker for recognition of an illness condition or a pathogen (Landegren242:229 [Science] (1988), Mikkelson(1996) Electroanalysis, 8:15-19). Various assays are daily performed in the microbiology laboratory or the pathology laboratory using such an approach.
[0006]
Generally in a molecule nucleic-acid HAIBUDAIZESHON, an antibody ligation reaction, a protein ligation reaction, and a lectin ligation reaction [whether it inserts (to for example, double helix of DNA) and] Or are detected by use of the indicator which is one of whether it is fixed to either a target or a probe molecule by covalent bond. (For example) SosnowskiProc(s)(1997).Natl.Acad.Sci.USA, 94:1119-1123, LePecq and Paoletti(1966) Anal.Biochem., 17:100-107, Kapuscinski And Skoczylas(1977) Anal.Biochem., 83:252 -257 reference. It is used in order that electrochemical luminescence may also detect the electrical activity luminescence marker inserted depending on the case (Pollard-KnightAnal(s) (1990).Biochem., 185:84-89, Pollard-KnightAnal(s)(1990).Biochem., 185:353-358, TizardProc(s) (1990).Natl.Acad.Sci.USA, 12:4514-4518). All of these detection strategies are either in front of the ligation reaction between a probe and a target molecule, or the back, and need derivatization of a target or a probe molecule (to for example, insertion or indirect indicator attachment sake). (to for example, covalent-labeling attachment sake) This brings about a contamination problem. Furthermore, when two or more analyte is analyzed by coincidence, two or more indicators must be used. Furthermore, complicated sample handling is required, and it increases the risk of contamination further, and it leads to/or the mistaken analysis. The above and other problems are conquered by this invention.
[0007]

(Epitome of invention)
This invention offers the new device and new approach of detecting and/or quantifying two or more analyte in a sample. This invention offers the flow through minute fluid (for example, capillary tube) biosensor which detects the target analyte (for example, nucleic acid) from which it differs in a sample, after combining with each one of affiliated "joint partners" (for example, a nucleic acid, an antibody, lectin, etc.). Generally, the section into which capillary tube channels differ [a joint partner "a probe" specific to various analyte] for example, using a photosensitive biotin / avidin technique is fixed. The flash plate of the sample is carried out into a capillary tube after that, consequently the target analyte combines with the joint partner (trapping agent) fixed by the capillary tube wall, and the remaining samples are eluted from a capillary tube. Finally, the analyte (it joined together) in which complex was formed is emitted along with the overall length of a channel, it passes a detector and a flash plate is carried out. In a desirable operation gestalt, the target-analyte which carried out desorption is detected using a sinusoid voltammetry in the copper electrode arranged down-stream (Singhal and Kuhr(1997) Anal.Chem., 69:3552-3557, SinghalAnal(s)(1997).Chem., 69:1662-1668). The time amount from elution of the target analyte to detection is used in order to determine the true character of each analyte. It is the molecule of the same kind (for example, wholly nucleic acid), or two or more analyte of a different kind (for example, protein and a nucleic acid) does in this way, and can diagnose using a single biosensor. The sensor is specific to altitude by a specific joint partner's use, and high sensitivity very much by electrochemical detection.
[0008]
Therefore, in 1 operation gestalt, this invention offers the device which detects two or more analyte in a sample. This device is set here including the channel to which each joint partner of two or more analyte is being fixed. When each joint partner of two or more analyte is stationed to the field to which channels differ, the channel has the cross-sectional area small enough and the inside of a channel is emitted to the analyte by it from two or more joint partners at flowing fluid The analyte is spatially separated until it reaches the detection point which met the down-stream channel from the joint partner or its edge, and the detector which detects the analyte on the detection point.
[0009]
Channels may be all expedient channels, such as a capillary tube, capillary-electrophoresis tubing, a channel etched into the front face, and a channel formed with the non-dense liquor printed on the front face. A channel can essentially have all dimensions, as long as it fully continues dissociating so that it may be identified, when the analyte arrives at the detection field or channel edge in a channel. A desirable channel has the cross section which gives less than about one Reynolds number (Re). a desirable channel -- about 500 micrometers or less -- more -- desirable -- about 100 micrometers or less -- it has the cross-section diameter or width of face of about 50 micrometers or less most preferably. an especially desirable device -- setting -- two or more target analyte -- at least 3 -- desirable -- at least 4 -- at least 5 and the analyte (and joint partner from whom the large number so differ) from which at least 10, at least 50, at least 100, or at least 500 differs most preferably are included more preferably. Although not restricted to below, a variety of joint partners including an antibody, binding protein, and a nucleic acid are suitable. Similarly, many detectors are suitable and a spectrophotometer (for example, absorbance spectrophotometer) and (all amperometries, a voltammetry, the potential difference, and/or a coulometric-analysis detector are essentially included) a electroanalysis-detector are mentioned. A voltameter, especially a sinusoid voltameter are mentioned as a desirable detector.
[0010]
In another operation gestalt, this invention offers the approach of detecting two or more target analyte in a sample. This approach the fluid containing the process;ii sample which offers the detection device indicated in this specification A channel is passed under the conditions which the target analyte which exists in a fluid combines with each one of joint partners, respectively. The process which detects the analyte in the location which met the down-stream channel from the process;iv joint partner who emits the analyte to the flow of the fluid passed along with the process;iii channel which codes the analyte spatially along with a channel by that cause from a joint partner is included. Indicator attachment of the

analyte is not carried out in a desirable approach. In a desirable operation gestalt, indicator attachment of the analyte is not carried out especially. an especially desirable device -- setting -- two or more target analyte -- at least 3 -- desirable -- at least 4 -- at least 5 and the analyte from which at least 10, at least 50, at least 100, or at least 500 differs most preferably are included more preferably (and so, the joint partner from whom the large number differ exists in the channel containing a detection device). Induction of the fluid flow is carried out by differential pressure and/or the electroendosmose style in some desirable operation gestalten. Fluid flow. As a "sample" fluid desirable for detection of the analyte, blood, plasma, a blood serum, urine, the liquid in the oral cavity, cerebrospinal fluid, and lymph are mentioned. Detection can be based on various approaches including a spectrophotometer (for example, absorbance spectrophotometric analysis) and (all amperometries, a voltammetry, the potential difference, and/or coulometric analysis are essentially included) the electroanalysis-approach. The desirable detection approaches are a voltammetry, especially a sinusoid voltammetry. Especially, in a desirable approach, the analyte is a nucleic acid and detection detects the target analyte by the concentration below 1x10-9M.

[0011]

(Definition)

In this specification, the vocabulary "a polypeptide", a "peptide", and "protein" are used possible [transposition], in order to point out the polymer of amino acid residue. These vocabulary is applied not only to the amino acid polymer which is the artificial chemical analog of the amino acid which exists in the nature to which one or more amino acid residue corresponds but the amino acid polymer which exists naturally.

[0012]

As the vocabulary "an antibody" is used in this specification An intact immunoglobulin, A Fv fragment only including the variable region of a light chain and a heavy chain, the Fv fragment combined by the disulfide bond (BrinkmannProc(s)(1993).Natl.Acad.Sci.USA, 90:547-551), Fab or (Fab) '2 fragmentation containing the parts of a variable region and a constant region, The antibody containing a single strand antibody etc. by which various gestalten were embellished or changed is included (Huston et al. [BirdScience(s)(1988) 242:424- 426;] (1988) Proc.Nat.Acad.Sci.USA 85:5879 -5883). An antibody may be an animal (especially a mouse or a rat) or the Homo sapiens origin, or may be a chimera (Morrison et al. (1984) Proc Nat.Acad.Sci.USA 81:6851 -6855) or hominization (JonesNature (s)(1986) 321:522-525; and open British Patent application #8707252).

[0013]

The member of the vocabulary "a joint partner", a "trapping agent", or a "joint pair" says other molecules and the molecule combined specifically, in order to form junctional complexes, such as an antibody-antigen, a lectin-carbohydrate, a nucleic-acid-nucleic acid, and biotin-avidin. In a desirable operation gestalt, association is mainly especially materialized by the noncovalent bond (for example, ion, canal) interaction.

[0014]

when pointing out biomolecules (for example, protein, a nucleic acid, an antibody, etc.) so that it may be used by this detail letter, the ligation reaction which determines existence of the biomolecule in the different-species ensemble of a molecule (for example, protein and other biologicalses) is said [vocabulary / "it joins together specifically"]. Therefore, specific ligand or a specific antibody is combined with the specific "target" molecule under the specified conditions (for example, the immunoassay conditions in the case of an antibody or the stringent hybridization conditions in the case of a nucleic acid), and it does not join together in other molecules which exist in a sample, and a significant amount.

[0015]

The vocabulary "a channel" says the path which draws the flow of a fluid in the specific direction. A channel can be formed as the slot which has a pars basilaris ossis occipitalis and a flank, a trench, or "tubing" surrounded completely. With a part of operation gestalten, a channel does not have even the need of having a "flank." For example, a hydrophobic polymer can be applied to a flat front face, and the

flow of the fluid in the front face can be restricted and/or guided in the narrow (for example, hydrophilic property) range by it. A channel is preferably equipped with at least one front face where joint partner (capture) drugs may be fixed.

[0016]

The "target analyte" is all the units or two or more molecules which should be detected and/or quantified in a sample. As desirable target analyte, biomolecules, such as a nucleic acid, an antibody, protein, and a saccharide, are mentioned.

[0017]

The vocabulary "a micro channel" is used about the channel which has the dimension which enables low Reynolds number actuation (Re<= 1, preferably Re<= 0.1, more preferably Re<= 0.01, most preferably Re<= 0.001) in this specification. Generally low Reynolds number actuation and hydrodynamics are governed by viscous force rather than inertial force.

[0018]

A vocabulary capillary tube (capillary tube) says tubing (for example, generally the flow of low Re is given) of a narrow dimension. Generally an open end capillary tube sucks up water by capillary action, when water is contacted. Although a capillary tube is not restricted to below, it can be manufactured with many ingredients containing glass, plastics, a quartz, a ceramic, and various silicates.

[0019]

"Capillary-electrophoresis tubing" says [in / therefore / a capillary-electrophoresis device] a design and/or the "capillary tube" which is generally used, or was meant so that it might be used.

[0020]

The vocabulary "a nucleic acid", an "oligonucleotide", or at least two nucleotides by which the equivalent phrase was combined with one by covalent bond in this specification are said grammatically. Although the nucleic acid of this invention is a single strand or a double strand preferably and generally includes a phosphodiester bond, so that it may outline below depending on the case For example Phospho RUAMIDO (BeaucageTetrahedron(s) (1993)) 49 (10):1925 and bibliography;Letsinger(1970) J.Org.Chem.35:3800; -- SprinzlEur(s)(1977).J.Biochem.81:579;LetsingerNucl(s)(1986).Acids Res.14:3487; -- Sawai et al. (1984) -- Chem.Lett.805 and LetsingerJ(1988).Am.Chem.Soc.110:4470; -- and PauwelsChemicaScripta(s) (1986) 26:141 9 Phosphorothioate (MagNucleic(s)(1991) Acids Res.19:1437; and U.S. Pat. No. 5,644,048), Phosphorodithioate (Briu et al. (1989) J.Am.Chem.Soc.111 : 2321), O-methyl phosphoroamidite (O-methylphophoroamidi te) association () [Eckstein, Oligonucleotides and Analogues:A Practical Approach,] [Oxford] University Refer to Press, And a peptide nucleic-acid frame And association () [Egholm] (1992) J.Am.Chem.Soc.114:1895; -- Meier et al. (1992) -- Chem.Int.Ed.Engl.31:1008;Nielsen(1993) Nature -- 365:566;CarlssonNature(s) (1996) The nucleic-acid analog including 380:207 reference which may have a mutual frame is contained. Other similar nucleic acids An electropositive frame (Denpcy et al. (1995) Proc.Natl.Acad.Sci.USA 92:6097), a nonionic frame (U.S. Pat. No. 5,386,023 and 5,637,684 --) 5,602,240, 5,216,141 And 4,469,863;Angew. Chem.Intl.Ed.English (1991) 30:423;Letsinger(s) (1988) J .Am.Chem.Soc.110:4470;Letsinger(s) (1994) Nucleoside & Nucleotide 13:1597; -- Chapter 2 -- and ASC Chapter 3 Symposium Series 580, "Carbohydrate Modifications in Antisense Research", Y.S.Sanghui, and P.Dan The volume on Cook; [Mesmaeker et al. (1994),] Bioorganic & Medicinal Chem.Lett.4:395;JeffsJ(1994).Biomolecular NMR 34:17;Tetrahedron Lett.37:743 (1996), And U.S. Pat. No. 5,235,033, 5,034,506, the ibid of Chapter 6, and Chapter 7 () [ASC Symposium Series 580,] ["Carbohydrate Modifications] in Antisense Research", Y.S.Sanghui, and P.Dan A thing equipped with the non ribose frame which contains the thing of a publication in the volume on Cook is included. The nucleic acid containing one or more carbocyclic saccharides is also contained in the inside of a definition of a nucleic acid (refer to Jenkins et al. (1995), and Chem.Soc.Rev.169 -176 pages). Some nucleic-acid analogs are indicated by Rawls (Rawls, C&E News Jun.2, 1997 or 35 pages). These qualification of a ribose-phosphate frame can be performed in order to make addition of an additional part called an indicator easy or to increase the stability and the half-life of the molecule concerned in a physiological environment.

the vocabulary -- "-- ** -- it hybridizes specifically -- " -- and "specific hybridization" -- and -- "-- ** -- it hybridizes alternatively -- " -- alternative association of a nucleic-acid molecule to a specific nucleotide sequence, doubleness, or high buri die JINGU is said under stringent conditions as used by this detail letter. A probe hybridizes the vocabulary "stringent conditions" on the target sequence and selection target, and whether it being made extent with few other arrays and the conditions which fitted in again and which are not a comb are said. the stringent hybridization and the stringent hybridization washing conditions in a situation of nucleic-acid hybridization -- an array -- it is anaclitic and differs under a different environmental parameter. Comprehensive guidance of nucleic-acid hybridization For example, Tijssen(1993) Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes Section I, Chapter 2, and Overview of principles of hybridization and the strategy of nucleic acid probe It is found out by assays, Elsevier, and N.Y. (Tijssen). Generally, highly, by regular ionic strength and pH, stringent hybridization and stringent washing conditions are chosen so that lower about 5 degrees C than the thermal melting point (Tm) of a specific array. Tm(s) are the probe with which 50% of the target sequence agreed completely, and temperature to hybridize (under regular ionic strength and pH). Very stringent conditions are chosen so that equally to Tm about a specific probe. An example of the stringent hybridization conditions for the hybridization of a complementary nucleic acid which has the complementary residue which exceeds 100 in an array or a filter in Southern blotting or a Northern blot A standard hybridization solution It uses and is 42 degrees C (for example). [one to three Sambrook(1989) Molecular Cloning:A Laboratory Manual(s) (the 2nd edition), Cold Spring Harbor Laboratory,] [Cold] Spring Harbor Reference and hybridization are performed in all night in Press, NY, and the following detailed explanation. An example of stringent washing conditions is 0.15M [for about 15 minutes and 72 degrees C] highly. It is NaCl. In the case of the item of the SSC buffer solution, an example of stringent washing conditions is 0.2xSSC washing at 65 degrees C for 15 minutes (for example, refer to above-shown Sambrook). Mostly, in order to remove a background probe signal, low stringency washing precedes with high stringency washing. For example, an example of stringency washing of whenever [for doubleness of the nucleotide exceeding 100 / middle] is 45-degree C 1xSSC for 15 minutes. For example, examples of low stringency washing for doubleness of the nucleotide exceeding 100 are 40 degrees C 4x - 6xSSC for 15 minutes.

[0021]

A difference of localization of concentration distribution of the molecule (for example, analyte) of two or more kinds [in / in "spatial separation" / a fluid stream] is said. When the analyte is separated spatially (that is, flow coding was carried out), even if the type of all the signals of the analyte is the same, it will be possible to detect the signal according to individual of each target analyte. Therefore, the location or time amount along "passage" of detection can determine the true character of the analyte, and the difference of an indicator related to each analyte is not required.

[0022]

The electroanalysis-approach says the approach of using a system or the "electric" properties (for example, resistance, conductance, capacitance, an impedance, etc.) of the analyte, in order to take out the information about the system. As the electroanalysis-approach, all amperometries, a voltammetry, the potential difference, and/or the coulometric-analysis approach are essentially mentioned. As the desirable electroanalysis-approach, cyclic voltammetry, an alternating current, a direct current or a rotation ring disk voltammetry, a sinusoid voltammetry, impedance spectroscopy, etc. are mentioned.

[0023]

The vocabulary "cyclic voltammetry" or an "aging voltammetry" is used possible [transposition], in order to point out cyclic voltammetry. The vocabulary "a sinusoid voltammetry" is used in order to point out cyclic voltammetry generally (for example, based on one containing a square wave, a triangular wave, etc. of aging electrical potential differences although not restricted to below), or in order to point out the use of a large amplitude sine wave potential wave used for U.S. Pat. No. 5,650,061 in a mode similar to cyclic voltammetry as a publication.

[0024]

(Detailed explanation)

(I. The efficient detection approach of two or more analyte)
This invention offers the new approach and new machine for quick detection of two or more analyte in a sample, and/or quantification. In 1 desirable operation gestalt, this invention contains the channel which fixed the specific joint partner in it in the analyte expected detection. Since a different joint partner is stationed to the field to which channels differ, when the analyte is combined, they are coded by each one of locations which met the channel at "space target." The combined analyte is behind released from a joint partner, or the inside of a channel is emitted to a joint partner / analyte complex into flowing fluid from the wall of a channel. As [separate / spatially / the analyte / until the analyte reaches the detection point of a down-stream channel from the above-mentioned joint partner / as for the dimension of a channel / continue]
[0025]
If the analyte or the analyte / joint partner complex is emitted to flow, they will be coded spatially. That is, it depends for each one of locations to both streams on the location of a joint partner when they are being fixed to the channel wall. Therefore, the time difference between emission and detection can be used in order to identify specifically the specific (or it does not generate) analyte which generates an output signal.
[0026]
Since the analyte may be identified specifically, without using an indicator in order to distinguish them from other analyte respectively, a large number, redundant sample handling, and a labeling process are eliminated. This removes many labeling and contamination problems. Moreover, the risk of the sample contamination which may lead to an incidence rate with high false positivity is also reduced or eliminated.
[0027]
It is mentioned especially that it is exchangeable to the inside and outside of the device with which it can prepare good and various minute fluid structures (for example, channel) perform flow of sample handling and a fluid and analyte detection before a channel's using it. It can have according to the analyte of the set from which a different channel differs, and the same or two or more different channels may be performed by coincidence.
[0028]
Therefore, the approach and device of this invention fit detection of the analyte in a clinical environment good. The capacity to detect unguided object-ized analyte (for example, DNA, mRNA, etc.) simplifies a procedure remarkably, and supports sample contamination and the mistaken prevention of the problem of discernment.
[0029]
Use of the copper electrode according [on 1 especially desirable operation gestalt and] to a cyclic (for example, sinusoid) voltammetry conquers many of problems which the conventional electrochemical measuring method encounters, and enables detection of the analyte by it. The high sensitivity of the detection strategy originates in the effective decoupling of the faraday signal from the capacitive background current in a frequency domain. It can follow, for example, ssDNA and dsDNA can be detected in a picomole concentration range, and an electrochemical signal originates in oxidation of the saccharide which can be accessed easily [the periphery of a DNA double helix] compared with ssDNA of the same size.
[0030]
The sensor which can detect two or more targets only using one detector offers a cheaper and small detection system also with easy manufacture.
[0031]
(II. system component)
(A) Channel
(1) The type and dimension of a channel
A channel is suitable for operation of this invention also by what type of channel as a matter of fact, as long as passage of the matter inside a channel is enabled without being accompanied by essential mixing

between the components in a solution in a different location which met the channel. That is, it is spatially continued by separating [the "down-stream" detection point] the analyte (or reagent in which other detection is possible) emitted first in the location according to individual which met the channel from the initial emission point in a desirable capillary tube. Even if, even if the type of the signal about all of analyte is the same, the capacity for the signal according to individual of each target analyte to be detectable is called spatial separation. Therefore, the time amount of the location along "passage" or detection can determine the true character of the analyte, and the difference of an indicator related to each analyte is not required.

[0032]

However, spatial separation does not require the perfect separation between analyte. Considerable overlap can be existed on the contrary, peak concentration can be detected, and a related concentration profile is measured and/or calculated and can give a positivity / electronegative detection, and/or perfect analyte quantification.

[0033]

A channel especially desirable to use by this invention is a "micro channel." The vocabulary "a micro channel" is used about the channel which has the dimension which enables low Reynolds-number actuation, i.e., the thing by which the dynamics of a fluid is governed by viscous force rather than inertial force, in this specification. The Reynolds number called ratio of inertial force to viscous force by the way is given by the following.

[0034]

Re=rho d2-/eta tau+rho ud/eta

As for a velocity vector and rho, fluid density and eta of u are time scales from which, as for the viscosity of a fluid, and d, the property dimension of a channel changes, and, as for tau, a rate changes here (being here u/tau=delta u/dt). The vocabulary "a property dimension" is here and is used as everyone knows about the dimension which determines the Reynolds number by this work. In the case of a cylindrical shape channel, it is a diameter. In the case of a rectangle channel, it is fundamentally dependent on the smaller one of width of face and the depth. It means saying that it is dependent on the width of face of the crowning of "V" in the case of V typeface channel. Count of Re about various morphological channels can be seen in the standard textbook of hydrodynamics (for example, Granger (1995) Fluid Mechanics, Dover, N.Y.;Meyer(1982) introduction to Mathematical Fluid Dynamics, Dover, N.Y.).

[0035]

The behavior of the flow of the fluid in a steady state (tau->infinity) is characterized by Reynolds number Re=rho ud/eta. The hydraulic system by which micro processing was carried out is in a low Reynolds-number regime (Re is less than about one) mostly for small size and a low speed. In this regime, a turbulent flow and a secondary flow, therefore the inertia effectiveness of flowing and producing mixing inside can be disregarded, and viscous effectiveness governs dynamics. Generally under such conditions, the flow in a channel is stratified.

[0036]

Since a Reynolds number is dependent not only on a channel dimension but the time scale from which fluid density, fluid viscosity, a fluid rate, and a rate change, the absolute upper limit of a channel diameter is not specified clearly. According to the channel geometrical configuration actually designed good, the high processing ability system which can avoid about R< 1000 if it depends especially about R< 100, therefore has large channel size relatively is possible for a turbulent flow. Desirable channel property dimension range is about 0.5 micrometers thru/or 100mm. Especially a channel range with a property dimension of about 1 micrometer - about 100 micrometers is desirable, and about 5 micrometers - about 100 micrometers are the most desirable. More desirable range is about 5 micrometers thru/or 50 micrometers.

[0037]

The device of this invention does not need to be restricted to low Reynolds number actuation. a signal with different analyte mutual when a joint probe is estranged widely and the analyte so emitted is widely

estranged in flow -- "overlap ****" -- remarkable convective mixing may occur in a channel, without carrying out a mask. Furthermore, as long as remarkable mixing of two analyte may occur and remarkable (for example, it is statistically significant) space separation exists between the peak concentration of two analyte, he can distinguish a signal and it will be understood that detection of each analyte can carry out. However, quantification of the analyte according to each may become gradually more difficult as the analyte mixes each other. Nevertheless, even such a situation can obtain quantification by evaluating or modeling the spatial distribution of the analyte based on the location and the rate of fall-off of a concentration peak, in order to give approximation of an integral signal to each analyte.

[0038]

As long as above-mentioned mixed requirements are fulfilled as above-mentioned, all channel configurations are proper. Therefore, although not limited to a suitable channel below, the channel formed of an obstruction [which counters], open slot, and closed ditch etc. is included. As for a channel, the shape of the shape of circular, a rectangle, a rectangle, a triangle, and v character and u character, a hexagon, an octagon, an irregular form, etc. can have all cross sections as a matter of fact. The channel used in this invention does not need to be continuous. It can follow, for example, a channel can be formed by the aggregate, a copolymer, or cross linked polymer of a porous particle etc.

[0039]

As long as the ingredient is essentially stable to the solution which passes through the inside of it, all channel ingredients fit operation of this invention. or [that a desirable ingredient is combinable with a joint partner] -- or as it joins together, can derivatize or it is a joint partner's linker. Furthermore, in a desirable operation gestalt, an ingredient is chosen and/or reformed so that it may not combine with the analyte substantially. Moreover, it does not combine with a probe in the field besides the reason expected to fix a probe, or a desirable ingredient does not interact to another appearance.

[0040]

Although especially a desirable ingredient is not limited to below, it contains glass, silicon, a quartz or other minerals, plastics, the ceramics, a metal, paper, a metalloid, a semi-conductor, cement, etc. Furthermore, the matter which forms gels, such as protein (for example, gelatin), a lipopolysaccharide, a silicate, agarose, and polyacrylamide, can be used. A variety of organic polymers and inorganic polymers of nature and both composition may be used as an ingredient on the front face of a solid-state. An instantiation-polymer contains polyethylene, polypropylene, Pori (4-methylbutene), polystyrene, polymethacrylate, Pori (ethylene terephthalate), rayon, nylon, Pori (vinyl butyrate), poly vinylidene JIFURUORIDO (PVDF), silicon, polyformaldehyde, a cellulose, cellulose acetate, a nitrocellulose, etc.

[0041]

In the case of conductivity or a semi-conductive substrate, an insulating layer exists in a substrate preferably. This is especially important when a device incorporates an electro-technical element (for example, the direction system of an electric fluid, a sensor, etc. move an ingredient around a system using the electroendosmose force). the application for which, as for a substrate ingredient, they are meant in the case of a polymer substrate -- responding -- hard, half rigidity or non-hard one, and opacity -- suppose that it is translucent or transparent. For example, it is manufactured by the transparent material optically partially [in order that the device containing a visual-detection element may enable the detection or may generally support it at least] at least. Or glass or the transparent aperture of a quartz may be taken in by the device about the detecting element of such a format again. Additionally, a polymer ingredient has a straight chain or a branching principal chain, and a bridge is constructed over it or it can presuppose un-constructing a bridge to it. Especially the example of a desirable polymer ingredient contains for example, poly dimethylsiloxane (PDMS), polyurethane, a polyvinyl chloride (VPC), polystyrene, polysulfone, a polycarbonate, etc.

[0042]

A channel can be used as the component of a larger body. Therefore, a channel can be assembled with other one or more channels, in order to obtain many channels, and assay from which plurality differs by it can be performed to coincidence. A channel can be used as the component of a machine including

suitable liquid handling, detection and/or sample handling / application function.
[0043]
moreover, a channel can carry out "plug-in" to the machine which performs assay of this invention suitably -- it can manufacture as a unit of reusable or throwing away. Although a channel is not limited to below, it is understood that it can prepare or more for any one in a variety of bodies containing a micro titration pan (for example, PVC, polypropylene, or polystyrene), a test tube (glass or plastics), dip sticks (for example, glass, PVC, polypropylene, polystyrene, a latex, etc.), a micro centrifuge tube or glass, a silica, plastics, a metal, or a polymer bead.
[0044]
With a desirable operation gestalt, one or more channels are especially manufactured as an element of the "integrated circuit" which is prepared in glass or a silicon slide as a capillary tube channel, or has an onboard circuit element for control of liquid flow, application of a sample, and/or detection of a signal as capillary tube tubing (for example, capillary-electrophoresis tubing). In the most desirable operation gestalt, as illustrated in the example in this specification, it has a channel as capillary tubes, such as capillary-electrophoresis tubing.
[0045]
(2) Channel manufacture
The approach of manufacturing the channel of this invention is well-known to this contractor. For example, when a channel is formed from one or more capillary tubes, a capillary tube is purchased from a commercial contractor (for example, Polymicron Technologies, Tucson, Az), or by the conventional capillary tube "drawing" **, it can draw out or extrude and it can be carried out.
[0046]
When manufacturing a channel on a front face, they can be formed by standard technique, for example, machining, shaping, sculpture, etching, a laminating, extrusion, or deposition is possible for them.
[0047]
In 1 desirable operation gestalt, a channel is manufactured using a well-known micro-machining process (for example, photolithography) in solid-state electronic industry. Usually, a micro device, for example, a micro channel, is created in the form of the semiconductor wafer used in order to manufacture an integrated circuit from a semi-conductor substrate called extensively available crystal silicon or glass. Manufacture of the micro device from a semiconductor wafer substrate can utilize a broad experience of both the surface etching technique developed by the semi-conductor processing industry for integrated-circuit (IC) manufacture, and bulk etching technique for the similarity of an ingredient.
[0048]
In order to create a movable element, surface etching used in order to form a thin surface pattern in a semiconductor wafer in IC manufacture is correctable so that sacrifice undercut etching of the thin layer of a semiconductor material may be enabled. Bulk etching used in case a deep trench is generally formed in a wafer using an anisotropic etching process in IC manufacture can be used in order to machine an edge or a trench to a precision in a micro device. In order to remove the ingredient by which a mask is not carried out from a wafer, "wet processing" which uses chemicals called a pottasium hydroxide solution can perform both surface etching and bulk etching of a wafer. In order to form various channel elements in micro device creation, it is even possible to use the anisotropy wet processing technique which depends on the distinctive crystal orientation of an ingredient, or is dependent on use of an electrochemical dirty stop.
[0049]
Generally another etching processing technique which allows the considerable freedom of a micro device design is known as "dry etching processing." Especially this processing technique is suitable for the anisotropic etching of the fine structure. Dry etching processing contains many gaseous phases or plasma phase etching technique which attains to even a little isotropic low energy plasma technique which guides the plasma stream which contains chemical reactivity ion in order to carry out induction of the formation of an volatile resultant to a wafer from the high anisotropy sputtering process which carries out the impact of the wafer with a high energy atom or ion in order to move a wafer atom to a

gaseous phase (for example, ion beam milling).
[0050]
There is an especially useful dry etching process known as reactive ion etching in the middle of high energy sputtering technique and low energy plasma technique. Reactive ion etching is accompanied by guiding an ion content plasma stream to a semi-conductor or other wafers for instantaneous sputtering and plasma etching. Reactive ion etching holds some of profits of an anisotropy related to sputtering, though reactant plasma ion is offered for formation of the gaseous-phase-reaction product which answered contact of reactant plasma ion with a wafer. The rate of wafer ingredient removal is actually remarkably reinforced to either the sputtering technique performed independently or low energy plasma technique. Therefore, reactive ion etching has possibility of becoming the etching process which excelled for micro device creation by the ability of a high anisotropy etching rate being maintained relatively. An above-mentioned micro-machining technique is well-known to this contractor like many other things (for example, refer to Choudhury(1997) The Handbook of Microlithography, Micromachining, and Microfabrication, Soc.Photo-Optical Instru.Engineer, and Bard & Faulkner(1997) Fundamentals of Microfabrication). Furthermore, the example of use of the micro-machining technique in silicon or a borosilicate glass chip can be seen to U.S. Pat. No. 5,194,133, 5,132,012, 4,908,112, and 4,891,120.
[0051]
In 1 operation gestalt, in a silicon (100) wafer, in order to carry out pattern formation of a channel and the connection, a standard photolithography technique is used for a channel and micro processing is carried out. In order that ethylenediamine and a pyrocatechol (EDP) may be used for two-step etching and may give a closed liquid system, anode plate junction of the Pyrex (trademark) (Pyrex) 7740 cover plate can be carried out in the field of silicon. In this case, liquid connection can be made behind silicon.

[0052]
In a desirable operation gestalt, a channel can be manufactured from other capillary tubes, such as glass, a quartz, or capillary-electrophoresis tubing, as above-mentioned.
[0053]
With other operation gestalten, in order that a channel may form a channel wall, by making a substrate deposit an ingredient, it can manufacture (using sputtering or other joining techniques), or casting/shaping of a channel may be done in an ingredient. Although casting / shaping channel is not restricted to below, it is easily manufactured from a variety of ingredients containing various metals, plastics, or glass. In a specific desirable operation gestalt a channel Various elastomers for example, alkylation chlorosulfonated polyethylene (Acsium (trademark)) -- A polyolefine elastomer (for example, Engage (trademark)), Chlorosulfonated polyethylene (for example, Hypalon (trademark)), A perfluoro-elastomer (for example, Kalrez (trademark)), neoprene polychloroprene, Casting is carried out with an ethylene-propylene-diene terpolymer (EPDM), chlorinated polyethylene (for example, Tyrin (trademark)), and various siloxane polymers (for example, poly dimethylsiloxane etc.).
[0054]
(B) Joint partner
one or more pieces by which the channel used by this invention was fixed to one or more front faces in the desirable operation gestalt -- biological -- a "joint partner" is held. biological -- the constituent of a "joint partner" or a "joint pair" says other molecules, the molecule combined specifically, or a presentation, in order to form junctional complexes, such as an antibody-antigen, a lectin-carbohydrate, a nucleic-acid-nucleic acid, and biotin-avidin.
[0055]
when pointing out biomolecules (for example, protein, a nucleic acid, an antibody, etc.) so that it may be used by this detail letter, the ligation reaction which determines existence of the biomolecule different-species ensemble of protein and other biologicalses is said [vocabulary / "it joins together specifically"]. Therefore, appointed ligand or an appointed antibody is combined with the specific "target" (for example, protein or a nucleic acid) under the specified conditions (for example, the

immunoassay conditions in the case of an antibody or the stringent hybridization conditions in the case of a nucleic acid), and it does not join together in other molecules and a significant amount.
[0056]
The joint partner used in this invention is chosen based on the target identified / quantified. It follows, for example, when a target is a nucleic acid, a joint partner is a nucleic acid or nucleic-acid binding protein preferably. When a target is protein, a joint partner is the receptor, the ligand, or the antibody preferably combined with the protein specifically. When a target is a saccharide or a glycoprotein, a joint partner is lectin etc. preferably.
[0057]
Although a proper joint partner (trapping agent) does not restrict to below, he contains a nucleic acid, protein, receptor binding protein, nucleic-acid binding protein, lectin, a saccharide, a glycoprotein, an antibody, a lipid, etc. Such a joint partner's composition or isolation approach is well-known to this contractor.
[0058]
(1) Preparation of a joint partner (trapping agent)
(a) Nucleic acid
The nucleic acid for using it as a joint partner in this invention can be manufactured or isolated according to either of the approaches of well-known a large number to this contractor. With 1 operation gestalt, a nucleic acid can consider as the isolated spontaneous generation nucleic acid (for example, genomic DNA, cDNA, mRNA, etc.). The method of isolating a spontaneous generation nucleic acid is well-known to this contractor (for example, refer to SambrookMolecular(s)(1989) Cloning-A Laboratory Manual (2nd edition), one to three-volume, Cold Spring Harbor Laboratory, Cold Spring Harbor, and N.Y.).
[0059]
However, in a desirable operation gestalt, a nucleic acid is created newly (de novo) by chemosynthesis. With a desirable operation gestalt, a nucleic acid (for example, oligonucleotide) An automatic composition device is used for Needham-VanDevanter and others (Needham-VanDevanterNucleic(s) (1984) Acids Res., 12:6159-6168) as a publication. Beaucage and Caruthers (it Caruthers(es) (1981) Beaucage and [ ] --) Tetrahedron According to the solid phase phospho lamination DAITO triester method which Letts. and 22 (20):1859-1862 indicated, it is compounded chemically. When required, generally purification of an oligonucleotide is performed by Pearson and Regnier (Pearson and Regnier (1983) J.Chrom.255:137-149) by either native acrylamide gel electrophoresis or the anion exchange HPLC as a publication. The array of an synthetic oligonucleotide can be checked using the chemical degradation method of Maxam and Gilbert (Maxam and Gilbert(1980) in Grossman and Moldave(piece) Academic Press, New York, Meth.Enzymol.65:499-560).
[0060]
(b) An antibody/antibody fragment
The antibody or antibody fragment for using it as a joint partner (trapping agent) It can manufacture by the approach of well-known many to this contractor. (For example) Harlow & Lane(1988) Antibodies:A Laboratory Manual, Cold Spring Harbor Laboratory, and Asai(1993) Methods in Cell Biology The 37th volume: Antibodies in Cell Biology, Academic Refer to Press and Inc.N.Y. It sets to one approach and an antibody is produced by making an animal (for example, rabbit) into immunity by the immunogen containing an epitope [hoping to recognize/capture]. It can be used in order that much immunogens may produce a specific reaction nature antibody. Recombination protein is immunogen desirable to production of a monoclonal antibody or a polyclonal antibody. Moreover, the protein which exists naturally can also be used by one of the pure or impure gestalten. A synthetic peptide is similarly created by standard peptide synthetic chemistry. for example, Barany and Merrifield, and Solid-Phase Peptide Synthesis; -- 3 - 284 pages The Peptides:Analysis, Synthesis, and Biology. -- the 2nd -- volume:Special Methods in Peptide Synthesis and Part A. -- [Merrifield] (1963) J.Am.Chem.Soc., 85:2149-2156, and Stewart(s) (1984) Solid Phase Peptide Synthesis, the 2nd edition, Pierce Refer to Chem.Co., Rockford, and Ill.

[0061]
The production approach of a polyclonal antibody is common knowledge at this contractor. If it says simply, the immunogen which is the cytoskeleton component refined preferably will be mixed with an adjuvant, and an animal will be made into immunity. Trial bleeding is performed for the immunoreaction to the immunogen pharmaceutical preparation of the animal, and it supervises by determining the reactant potency to a cytoskeleton component and a test presentation. When a high potency is obtained appropriately [the antibody to immunogen], blood is collected from an animal and antiserum is prepared. In a necessary case, in order to condense about a reactant antibody for a cytoskeleton component, the further fraction of antiserum can be performed. . (see the above-shown Harlow & Lane).
[0062]
This contractor can get a monoclonal antibody by the various techniques of concordance. If it says simply, generally immortalization of the spleen cell from the animal made into immunity with the desired antigen will be carried out by fusion to a myeloma cell (Kohler and Milstein(1976) Eur.J.Immunol.6:511 -519 reference). The alternate method of immortalization includes the transformation by other well-known approaches by the Epstein-Barr virus, the oncogene, the retrovirus, or this business. The yield of the monoclonal antibody which screens about an antigen the colony produced from a single immortalization cell for production of desired singularity and the antibody of an affinity, and is produced by such cell can be reinforced by various techniques including the impregnation to a vertebrate host's peritoneal cavity. Or it is also possible to isolate the DNA sequence which carries out the code of a monoclonal antibody or its joint fragmentation by screening a DNA library from a Homo sapiens B cell again according to the general protocol outlined by Huse and others (246:1275-Huse1281 [Science] (1989)).
[0063]
For example, production/selection also of antibody fragments, such as a single strand antibody (scFv or in addition to this), can be done using a phage display technique. The capacity which can discover an antibody fragment on the front face of the virus (a bacteriophage or phage) with which bacteria are infected makes it possible to isolate a single joint antibody fragment from the library of the uncombined clone exceeding 1010. In order to make an antibody fragment discover on the surface of phage (phage display), it is inserted in the gene to which an antibody fragment gene carries out the code of the phage surface protein (pIII), and antibody fragment-pIII fusion protein is displayed by the phage front face (Hoogenboom et al. [McCafferty / Nature / 348:552- 554;] (1990) (1991) Nucleic Acids Res. 19:4133 - 4137).
[0064]
Since the antibody fragment on the front face of phage is functionality, the phage holding an antigen joint antibody fragment can be isolated from uncombined phage with antigen affinity chromatography (348:552-McCafferty554 [Nature] (1990)). Depending on the affinity of an antibody fragment, a 1,000,000 times [20 times to] as many enrichment factor as this is obtained about one affinity sorting. However, more phage can be proliferated and one sorting can be made to already be received by infecting bacteria with the eluted phage. Thus, the 1000 times as many concentration by 1 time as this can increase 1,000,000 times in two sortings (348:552-McCafferty554 [Nature] (1990)). time [therefore,] concentration is low -- (-- MarksJ(1991).Mol.BioI.222:581-597) and affinity sorting of multiple times may bring about isolation of rare phage. Since sorting of the phage antibody library of an antigen produces concentration as a result, a large majority of clones combine an antigen after about three - four sortings. Therefore, it is necessary to analyze a small number of (hundreds) clone only about association with an antigen comparatively.
[0065]
By displaying various very large and V gene repertories on phage, a Homo sapiens antibody is not based on the conventional immunization, but can be produced (Marks et al. (1991) J.Mol.Biol. 222:581 -597). In 1 operation gestalt, natural VH and natural VL repertory which exist in a human peripheral blood lymphocyte were isolated from the non-immunizing donor by PCR. The splice of both the V gene

repertories was carried out at random using PCR, the scFv gene repertory was produced, the clone of this was carried out to the phage vector, and it produced the library of a 30 million phage antibody (this writing). The joint antibody fragment was isolated from this single "unsettled" phage antibody library to a different antigen exceeding 17 containing hapten, polysaccharide, and protein (Clackson et al. [Marks et al. / MarksJ(1991).Mol.Biol.222:581- 597; / (1993) .Bio/Technology. 10:779-783;GriffithsEMBO(s) (1993) J.12:725- 734;] (1991) Nature. 352:624 -628). The antibody was produced to the self-protein containing the thyroglobulin, an immunoglobulin, a human tumor necrosis factor, and human CEA (Griffiths et al. (1993) EMBO J.12:725 -734). Moreover, it is also possible by sorting out directly in a cell as it is to isolate the antibody to a cell surface antigen. The antibody fragment is very specific about the antigen used for sorting, and it has the affinity of the range of 1:M-100nM (Griffiths et al. [MarksJ (1991).Mol.Biol.222:581- 597;] (1993) EMBO J.12:725 -734). A bigger phage antibody library produces isolation of the antibody of twist a large number of the high joint affinity to the antigen of a larger rate as a result.

[0066]

(c) Binding protein

In 1 operation gestalt, a joint partner (trapping agent) may be binding protein. Although proper binding protein is not restricted to below, it contains a receptor (for example, cell surface receptor), receptor ligand, cytokine, a transcription factor and other nucleic-acid binding protein, a growth factor, etc.

[0067]

Protein can make mutation able to induce from the protein which isolated from the source of nature or was isolated, or can be compounded newly. A means to isolate the protein which exists naturally is well-known to this contractor. Although such an approach is not restricted to below, ammonium-sulfate precipitate, an affinity column, The well-known protein purification approach containing a column chromatography, gel electrophoresis, etc. is included (generally). R. Scopes and Protein (1982) Purification, Springer-Verlag, N.Y.;Deutscher(1990) Methods in The 182nd volume of Enzymology: Guide to Protein Purification and Academic Refer to Press and Inc.N.Y.

[0068]

When protein combines a target reversibly, the affinity column holding a target may be used in order to carry out affinity purification of the protein. Or it can rearrange with a HIS tag, and a target can be discovered, and protein can also be refined using a nickel2+/NTA chromatography.

[0069]

With another operation gestalt, protein may be chemically compounded using a standard chemical peptide synthesis technique. When a desired array is comparatively short, a molecule may be compounded as a single continuous polypeptide. When asking for a larger molecule, a partial array is compounded separately (in one or more units), and can be united by forming peptide linkage after that by the condensation of the amino terminus of one molecule, and the carboxyl terminus of the molecule of another side. This is performed using the same chemistry (for example, Fmoc, Tboc) as being typically, used in order to combine single amino acid in the peptide synthesis machine for commerce.

[0070]

After the C-terminal amino acid of an array is fixed to insoluble support, the solid phase composition accompanied by sequential addition of the remaining amino acid of an array is the approach that it is desirable for the chemosynthesis of the polypeptide of this invention. About the technique of solid phase composition Barany And Merrifield () [Barany] and Merrifield(1962) Solid-Phase Peptide Synthesis;3-284 page and The Peptides:Analysis and Synthesis -- Biology. 2nd volume:Special Methods in Peptide Synthesis, Part A., Merrifield et al. (MerrifieldJ(1963).Am.Chem.Soc., 85:2149-2156) -- and It is indicated by Stewart and others (StewartSolid(s)(1984) Phase Peptide Synthesis, the 2nd edition, Pierce Chem.Co., Rockford, Ill).

[0071]

In a desirable operation gestalt, it may be compounded using recombinant DNA methodology. Generally, this includes the process which reproduces protein, when still more nearly required, the process which creates the DNA sequence which carries out the code of the binding protein, the process

which puts DNA on the manifestation cassette under a specific promotor's control, the process which makes protein discover in a host, the process which isolates the discovered protein, and.
[0072]
DNA which carries out the code of the binding protein or the array of this invention For example, cloning of a suitable array and a limit Or Narang's and others phospho triester method (Narang et al. (1979) Meth.Enzymol. 68:90 -99), Brown's and others phosphodiester method (Brown et al. (1979) Meth.Enzymol. 68:109 -151), Beaucage's and others diethyl phospho lamination DAITO -- law (BeaucageTetra(s)(1981).Lett., 22:1859-1862) -- and It can prepare by the suitable approach including the direct chemosynthesis by the approach of the solid support method of U.S. Pat. No. 4,458,066 of the above arbitration.
[0073]
The nucleic-acid array which carries out the code of the desired binding protein may be discovered in the various host cells containing various high order eukaryotic cells, such as Escherichia coli, other bacteria hosts, yeast, COS and CHO, a HeLa-cell stock, and a myeloma cell system. A recombination protein gene may be connected possible [suitable about each host / an expression control array and actuation]. In the case of Escherichia coli, this contains the conclusion signal of an imprint as preferably as promotors, such as T7, trp, or lambda promotor, and a ribosome bond part. In the case of an eukaryotic cell, a regulatory sequence may include a splice donor and a receptor array preferably, including a promotor, the enhancer guided from an immunoglobulin gene, SV40, a cytomegalovirus, etc., and a polyadenylation array.
[0074]
A plasmid may be imported into the selected host cell by the well-known approach of the calcium phosphate processing or electric punching of a case of the calcium chloride transformation in the case of Escherichia coli, and a mammalian cell. The cell in which a transformation is carried out by the plasmid can be chosen by the resistance to the antibody given with the gene contained in plasmids, such as an amp gene, a gpt gene, a neo gene, and a hyg gene.
[0075]
Once it is discovered, recombination binding protein may be refined according to the protocol of the criterion of these above business.
[0076]
(d) A saccharide and a carbohydrate
A saccharide and a carbohydrate are included as other joint partners. A saccharide and a carbohydrate can be isolated from the source of nature, can be compounded with an enzyme, or can be compounded on a chemistry target. The path for production of specific oligosaccharide class formation is in. It is based on use of the enzyme (glycosyltransferase) which produces them by vivo. such an enzyme -- in of oligosaccharide REJIO for vitro composition -- it may be used as alternative and a stereo alternative catalyst (Ichikawa et al. (1992) Anal.Biochem. 202:215 -238). A sialyltransferase may be used in combination with an auxiliary glycosyltransferase. For example, the combination of a sialyltransferase and galactosyltransferase can be used. In order to compound desired oligosaccharide class formation, many approaches of using a glycosyltransferase are well-known. The instantiation-approach is indicated by WO 96/32491, Ito et al. (Ito et al. (1993) Pure Appl.Chem. 65:753), U.S. Pat. No. 5,352,670, No. 5,374,541, and No. 5,545,553. By being combined in an early reaction mixture, instead, an enzyme and a substrate can add the reagent of an enzyme and the 2nd glycosyltransferase circuit to a reaction medium, when the first glycosyltransferase circuit approaches completion. By carrying out two glycosyltransferase circuits in order in a single container, the whole yield improves rather than the protocol with which a middle kind is isolated.
[0077]
The approach of chemosynthesis is indicated by Zhang (ZhangJ(1999).Am.Chem.Soc., 121(4):734-753). If it says simply, the set of the base unit of the sugar base will be created by this approach by each base unit preloaded in a different protective group. A base unit is graded by the reactivity of each protective group. After that, it is correctly determined which fundamental component must be added to a reaction

so that the reaction of a single string [computer program] from the fastest thing to the thing of the maximum ** may produce a desired compound.
[0078]
(2) Adhesion of the joint partner to a channel
Many approaches of fixing biomolecule on various solid-state front faces are well-known in the field concerned. Covalent bond of the desired component is carried out, or it may be fixed by the noncovalent bond through specific or nonspecific association.
[0079]
When covalent bond is desired between a compound and a front face, a front face is polyfunctional or can usually carry out [many organic functions]-izing. The functional group which may be used for association may contain a carboxylic acid, an aldehyde, the amino group, a cyano group, ethylene, hydroxyl, a sulfhydryl group, etc. by existing in a front face. The approach of connecting various compounds with various front faces is common knowledge, and is illustrated by reference at abundance. For example, Ichiro Please refer to Chibata (Ichiro Chibata(1978) Immobilized Enzymes, Halsted Press, New York) and Cuatrecasas (Cuatrecasas, J(1970).Biol.Chem.245:3059).
[0080]
In addition to covalent bond, the various approaches of combining an assay component by the noncovalent bond can be used. Generally a noncovalent bond is nonspecific adsorption of the compound to a front face. Usually, a front face is blocked with the 2nd compound in order to prevent nonspecific association of an assay component by which the indicator was carried out. Or although it combines a front face with one component nonspecific, another thing is designed so that it may hardly join together. For example, with a carbohydrate content compound, the front face which holds lectin called concanavalin A will not be combined with the protein lacking in glycosylation by which the indicator was carried out, although it joins together. The various solid-state front faces for using it for immobilization by the noncovalent bond of an assay component are outlined in U.S. Pat. No. 4,447,576 and 4,254,082.
[0081]
A molecule is in when a joint partner is a nucleic acid or a polypeptide. It is chemically compoundable by situ. this permutes an optical instability protective group by the usual protective group (for example, dimethoxytrityl radical used in nucleic acid biosynthesis (DMT)) -- the standard chemosynthesis approach is included substantially. The exposure of the micro channel in the dispersed location produces alternative association with a monomer (for example, amino acid or a nucleotide), and the growth polypeptide of the irradiated part or a nucleic acid as a result. The approach of optical directivity polymer composition is common knowledge at this contractor (for example, U.S. Pat. No. 5,143,854, the PCT public presentation number WO 90/15070, WO 92/10092 and WO 93/09668, further 251,767 to 77 refer to [Science] the Fodor (1991)).
[0082]
In a desirable operation gestalt, a joint partner may be fixed using a linker (for example, gay 2 functionality or a hetero bifunctional linker). A linker proper in order to combine a biological joint partner is common knowledge at this contractor. For example, although protein or a nucleic-acid molecule is not restricted to below, it may be combined by either of the various linkers containing a peptide linker, a straight chain, or a branched-chain chain linker, or the heterocycle type carbon linker. A hetero bifunctional crosslinking reagent called the activity ester of N-ethyl malei mide it is used widely (Lerner et al. [for example,] (LernerProc(s)(1981).Nat.Acad.Sci.USA, 78:3403-3407) -- Kitagawa et al. (KitagawaJ(1976).Biochem., 79:233-236), Refer to Birch and Lennox (Birch and Lennox (1995) of Chapter 4, Monoclonal Antibodies:Principles and Applications, Wiley-Liss, N.Y.).
[0083]
With 1 desirable operation gestalt, a joint partner is fixed using a biotin / avidin interaction. In this operation gestalt, the biotin or avidin which has an optical instability protective group may be arranged at a channel. The exposure of the channel of the location according to individual produces association of the biotin to the channel in the location, or avidin. Then, the binder holding each biotin or avidin is

arranged at a channel, and it combines with each joint partner and is localized in an exposure part. It may be repeated in the location according to each where this process is wanted to fix a joint partner.
[0084]
Sigrist and others (SigristBio/Technology (1992), 10:1026-1028) has indicated the another suitable photochemistry-joint approach. By this approach, the interaction of ligand with an organic front face or an inorganic front face is mediated with the polymer which has the carbene which generates trifluoromethyl-aryl-JIAJIRIN which works as a linker molecule and which can be photoactivated. Photoactivation of the aryl-JIAJIRINO functional group in 350nm produces a highly reactive carbene, and covalent bond is attained by coincidence carbene insertion on both ligand and an inactive front face. Therefore, a reactant functional group is not required in which of ligand or the charge of supporting material.
[0085]
In the most desirable operation gestalt, in order to cover a fused silica front face with an organic coating, the thin layer of an epoxy resin (Epotek350) is applied to a fused silica (fused cilica) capillary tube (bore of 50 micrometers). A surface organic coating not only makes the minimum DNA adsorption in a capillary tube wall, but offers the polymerization-ized front face which can carry out direct immobilization of the DNA probe. Liu and others (Liu et al. (1996) J.Chromatogr. 723:157 -167) has indicated the protocol which covers a capillary tube front face with an epoxy resin. It dried under the nitrogen pressure of 20psi, and when saying simply, after carrying out the rinse of the capillary tube for 15 minutes with an acetone first, it was dried in oven at 100 degrees C for 1 hour. Epoxy resin 314ND (Epo-Tek, Billerica, MA) was dynamically covered on the capillary tube front face by attracting the acetone solution of epoxy resin mixture. The residual solvent was removed from the epoxy resin covering capillary tube by pouring nitrogen for 30 minutes at a room temperature. The bridge was constructed at 150 degrees C in 30 minutes and under the nitrogen pressure of after that 20psi by 80 degrees C in epoxy resin coating for 2 hours. The buffer solution washes the applied capillary tube for 30 minutes before use.
[0086]
Then, a specific DNA probe solution is poured into 1cm section of an epoxy resin covering capillary tube. In order that a DNA probe solution may combine a DNA probe with a capillary tube wall by the hydrophobic interaction and the electrostatic interaction, it is made to react with the piece of a capillary tube overnight. Other DNA probes are fixed like the piece of a covering capillary tube of same 1cm length. Once it is fixed by the capillary tube wall, after those hybridization fields' being deionized water and rinsing, the preparation assembled to the capillary tube biosensor which has a joint partner who is different in a different location will be completed.
[0087]
(C) The analyte detection approach
All approaches can be used according to the approach of this invention on the fact of biological molecule detection. Since the true character of various analyte is determined by those spatial positions in the flow which is moving the inside of a channel, there is no need for a different labeling system about each analyte. I hear that one advantage of this assay system, not to mention it, does not have the need of attaching an indicator in the analyte, and it is in it.
[0088]
The method of detecting the analyte is common knowledge at this contractor. When the analyte is labeled (for example, radioactivity, fluorescence, the MAG, or a mass indicator), the analyte is detected by detecting an indicator. However, with a desirable operation gestalt, the analyte is not labeled and it does not depend for the desirable detection approach on use of the indicator attached to the analyte. Although such a detection approach is not restricted to below, it includes detection of detection (for example, radiation and/or an absorption spectroscopy) of a lightwave signal, the electrical and electric equipment, and a magnetic signal, and detection of change of the electrical characteristics (for example, conductance/resistance, capacitance, an impedance, etc.) of the medium containing the analyte.
[0089]

With 1 simple operation gestalt, optical absorption of the fluid containing the analyte is supervised by the detector (for example, standard ultraviolet rays). However, a electroanalysis-detector is used with a desirable operation gestalt. With the most desirable operation gestalt, a electroanalysis-detector uses a voltammetry (for example, sinusoid).

[0090]

In a desirable operation gestalt, a sinusoid voltammetry includes supplying the analyte of the little purpose to a voltammetry electrode especially. A sine (or others carry out aging) electrical potential difference is applied to an electrode. The electrical potential difference which carries out aging (for example, sine) is the single period of a predetermined frequency, and in order to carry out the sweep of the formal potential of the target redox kind, it has the amplitude large enough. The response of the analyte to a sinusoidal voltage is alternatively detected by the higher harmonic of the fundamental frequency of an aging electrical potential difference. The method of performing an aging voltammetry is offered in the bibliography quoted U.S. Pat. No. 5,650,061 and in it.

[0091]

Although especially a desirable operation gestalt uses a sinusoid voltammetry, other voltammetry approaches are suitable for this invention good. As above-mentioned, especially the aging voltammetry approach is desirable, and such a voltammetry approach is not restricted to the use of an electrical potential difference in which a sine wave carries out aging. Moreover, other waves are suitable. Although such an approach is not restricted to below, it includes use of a square wave and a triangular wave (triangle wave). Such an aging voltammetry approach is common knowledge at this contractor (7 (1): for example, Cullison and Kuhr (1996) Electroanalysis, 1-6 reference).

[0092]

Discovery of this invention was that combination with the analyte isolation by which the code was carried out to sinusoid voltammetry detection and a space target provides altitude with detection/quantum of the specific analyte on very low level in a complicated sample (for example, blood serum).

[0093]

(III. integrated assay device)

The newest chemical-analysis system used in chemical manufacture, environmental analysis, a medicine diagnosis, and fundamental experiment room analysis has the capacity of full-automatic-izing preferably. Such a comprehensive analysis system (TAS) () [Fillipini] (1991) J.Biotechnol.18:153;Garn (s) (1989) Biotechnol .Bioeng.34:423;Tshulena(1988) Phys.Scr.T23:293;Edmonds(1985) Trends Anal.Chem.4:220 --;StinshoffAnal(s)(1985).Chem.57:114 R;Guibault(1983) Anal.Chem Symp.Ser.17:637;Widmer(1983) Trends Anal.Chem.2:8 perform automatically the function which attains to detection including transportation of the sample in a system, sample preparation, isolation, purification, data collection, and evaluation from installation of the sample to a system.

[0094]

In recent years, the sample preparation technique is reduced with the sufficient result to the miniaturized gestalt. Therefore, for example, a gas chromatography (Widmer et al. (1984) Int.J.Environ.Anal.Chem. 18:1), High pressure liquid chromatography (MullerJ(1991).High) Resolut.Chromatogr.14: 174;Manz (es) (1990) Sensors & Actuators Microcolumn edited (1985) by B1:249;Novotny Separations:Columns, Instrumentation and Ancillary Techniques J. Chromatogr.Library, Micro-Column edited (1984) by 30th volume;Kucera High Performance Liquid The volume Chromatography, Elsevier, and on Amsterdam;Scott (1984) Small Bore Liquid Chromatography Columns:Their Properties and Uses, Wiley, and N.Y.;JorgensonJ(1983). -- Chromatogr.255:335;KnoxJ(1979).Chromatogr.186:405; -- Tsuda et al. (1978) -- Anal.Chem.50:632 -- and capillary electrophoresis (ManzJ(1992).Chromatogr.593:253; -- Olefirowicz et al. (1990) -- Anal.Chem.62:1872;Second Int'l Symp.High-Perf.Capillary) Electrophoresis(1990) J.Chromatogr.516; -- Ghowsi et al. (1990) -- Anal.Chem.62:2714 are reduced with the sufficient result to the miniaturized gestalt.

[0095]

Similarly, it sets in another operation gestalt and this invention offers the integrated assay device (for

example, TAS) which detects and/or quantifies many analyte. An assay device contains a channel equipped with the joint partner fixed as above-mentioned. Furthermore, a desirable integrated assay device is one or more reservoirs which supply :detection system (for example, voltammetry system containing an electrode and/or related electronics), the buffer solution, and/or the Flushing fluid containing following one or more, a sample application well and/or an inlet, and a computer controller (control, such as a pump, a reservoir flow change, a detector, and a signal analysis system, sake).
[0096]
With a desirable operation gestalt, an integrated assay device equips a "removable" unit with a channel especially. When the capillary tube which follows, for example, can be easily inserted, and detached and attached from an accompanying device may be prepared in a module as a channel, thereby, a device can work easily by the assay of the set with which analyte differs.
[0097]
When the channel used with a device is tubing (for example, capillary-electrophoresis tubing), the conventional capillary-electrophoresis device is equipped with the subordinate piping and sample handling component, feeding component, and computer controller for the "integrated" assay device according to many this inventions. In order to offer the integrated assay device which fits detection and/or the quantum of various analyte good, it is hardly the need other than quite simple installation/addition of the detector (for example, sinusoid voltammetry detector) according to this invention, and related electronics.
[0098]
(Activation of IV. assay)
Generally, assay is performed by introducing a sample into the channel which has the fixed joint partner. A sample is kept suitable for the bottom of the condition each joint partner enables it to combine with the target analyte which may exist in a sample specifically. Then, the flash plate of the sample is carried out by installation of the buffer solution which supports emission of the analyte generally combined from a channel. The analyte emitted after that is detected by the down-stream detection point, and the true character of the analyte is determined by the time amount from emission to detection.
[0099]
(A) Preparation of a sample
as a matter of fact -- oh, a ** sample can analyze using this advantageous device and advantageous approach. However, with a desirable operation gestalt, a sample is a biological sample. The vocabulary "a biological sample" says the sample obtained from the component (for example, cell) of an organism or an organism so that it may be used in this specification. A sample can be taken as the thing of all biological organizations or a fluid. In many cases, a sample is a "clinical sample" which is a sample originating in a patient. Although such a sample is not restricted to below, it contains expectoration, cerebrospinal fluid, blood, a blood fraction (for example, a blood serum, plasma), a corpuscle (for example, leucocyte), an organization or a fine needle biopsy sample, urine, ascites and pleural effusion, or the cell originating in them. Moreover, a biological sample may also contain an organization intercept called the frozen section obtained for the purpose on histology.
[0100]
** of a biological sample (for example, blood serum) by which the direct method of analysis is carried out is also good, and before use by the assay of this invention, a certain preparation may be presented with them. Although such preparation is not restricted to below, it may include removal of the cell residual dust by suspension / dilution, or centrifugal separation of a sample in water or the suitable buffer solution etc., or selection of the specific fraction of the sample before analysis.
[0101]
(B) Feeding of the sample to a system
A sample can be introduced into the device of this invention according to the standard method of common knowledge to this contractor. By following, although a sample is used in a high-pressure liquid chroma TOGURAFISHI stem, it can be introduced into a channel through an inlet [like]. With another operation gestalt, a sample is applicable to the sample well connecting with a channel. In still more

nearly another operation gestalt, pump feeding of the sample may be carried out into a channel. The approach of introducing a sample into a channel is common knowledge, and a criterion in the technique of capillary electrophoresis and a chromatography.

[0102]

(C) Connection condition

A sample will be maintained at the basis of the conditions which promote specific association between a sample and a joint partner once it goes into a channel. The conditions which suit specific association between a joint partner and the analyte are common knowledge at this contractor. For example, the buffer solution suitable in order to promote association between an antibody and target protein is common knowledge in an immunoassay technique. for example, U.S. Pat. No. 4,366,241 -- No. 4,376,110 No. 4,517,288 and No. 4,837,168; Asai(1993) Methods in Cell Biology Volume 37:Antibodies in Cell Biology and Academic Press, Inc.New York;Stites & Terr(1991) Basic and Clinical Immunology 7th Refer to Edition. Similarly, the conditions at the time of a nucleic acid hybridizing specifically mutually are also common knowledge at this contractor (refer to above-shown Tijssen (1993)). A specific connection condition is optimized by this contractor about the specific set of a joint partner and the target analyte according to a well-known standard method. for example, Above-shown Tijssen (1993) and U.S. Pat. No. 4,366,241 -- No. 4,376,110, No. 4,517,288 and No. 4,837,168; Asai(1993) Methods in Cell Biology Volume 37:Antibodies in Cell Biology and Academic Press, Inc.New York;Stites & Terr(1991) Basic and Clinical Immunology 7th Refer to Edition.

[0103]

(D) Emission conditions

They are emitted after the analyte in a sample is specifically combined with the joint partner who fixed to the channel. Emission is suitably performed by the temperature conditions which contact a joint partner / analyte complex to the buffer solution and which are caused especially or destroy the interaction of a joint partner / analyte. Such a meeting may be destroyed by use of an elevated temperature, modifiers (for example, a urea, a formamide, etc.), quantity or low pH, quantity or low-salt, and various chaotropic agents (for example, guanidine hydrochloride) according to the pair of specific analyte / joint partner.

[0104]

(E) Analyte/flow in a channel

A sample, and/or a carrier / buffer-solution fluid can be introduced to a channel according to the standard approach, and can move the inside of/or a channel. For example, a fluid may be introduced and moved into a channel by the simple gravity feed from a "reservoir." Or the inside of a channel may be moved to a fluid by the pressure to the fluid pressure, and the deformable chamber/diaphram produced with either gas pressure or the various suitable pumps (for example, a peristaltic pump, a measuring pump, etc.) etc. again. Moreover, the inside of a channel may be moved also to the analyte by the electroendosmose approach.

[0105]

(F) Detection

Analyte detection can be based on either of many approaches of common knowledge to these above contractors as above-mentioned. In the desirable operation gestalt, the electrochemical detection approach was used and detection is based on the sinusoid voltammetry with the most desirable operation gestalt.

[0106]

The protocol for performing a sinusoid voltammetry is already indicated (Singhal et al.(1997) Anal.Chem.69:4828-4832; and U.S. Pat. No. 5,650,061). If it says simply, digital generation of the sine wave of 2Hz, 0.7 ****-p, and +0.35V direct current offset will be carried out using a software program. This sine wave is committed as impression potential to a copper electrode. The current responses from an electrode are collected by software on real time between the single overall lengths of an elution run. This time amount domain current response is changed into a frequency domain by the fast Fourier transform after that. The protocol for analyzing frequency spectrum is mentioned already (Singhal et al.

(1997) Anal.Chem.69:1662-1668). The spectrum corresponding to the analyte is obtained after background subtraction and a digital phase lock as stated above (above-shown Singhal et al. (1997)).
[0107]
(V. Kit for two or more analyte detection)
In 1 operation gestalt, this invention offers the kit screened in order to identify or quantify many the existence of the analyte or the absences in a sample. A kit contains the channel of this invention holding the various joint partners fixed on the surface of each one as it is shown in this specification. a channel may be designed for the simple and quick nest to an one apparatus assay device called a device equipped with the computer control system for control of analysis of suitable piping for maintenance of management of an electrochemical detector (for example, sinusoid voltammetry) circuit and a sample, and the flow of the fluid in a channel and application of a sample, the flow of a fluid, and a signal output as explained for example, to this detail in the letter. A kit can contain further the suitable buffer solution for use, other solutions, and the standard substance in the assay approach described into this specification.
[0108]
Furthermore, a kit may contain teaching materials including the directions (namely, protocol) for enforcing the approach of this invention. Although teaching materials generally contain a document or printed matter, they are not restricted to such a thing. Such directions are stored and all the media that can transmit them to an end user are taken into consideration by this invention. Although such a medium is not restricted to below, it contains an electronic storing medium (for example, a magnetic disk, a tape, a cartridge, a chip), an optical medium (for example, CD-ROM), etc. Such a medium may include the address to the Internet site which offers such teaching materials.
[0109]

[Translation done.]

EXAMPLE

(Example)
The following examples are not for showing in order to illustrate the invention in this application, and limiting.
[0110]
(Example 1)
(Electrochemical detection of the nano liter volume of DNA hybridization)
(An ingredient and approach)
(Reagent)
After deionizing the water to be used, it passed the Milli-Q water purification system (Millipore Corp., Bedford, Mass.). About tuberculosis (TB), a biotin-ized DNA probe specific to identification of a human immunodeficiency virus (HIV), and a cDNA target, it is Genemed. Special order composition was carried out through Synthesis and Inc. (San Francisco, Calif.) (Table 1). The DNA probe solution was produced by diluting 100microg [/ml] solution of the DNA probe dissolved in deionized water into 1:1 mixture with a DNA binding solution (Pierce Chemicals, CA). This joint solution makes it easy to combine DNA with a polymerization-ized front face by the canal and the electrostatic interaction. The fused silica capillary tube (Polymicron Technologies, Inc., AZ) was used in order to produce a capillary tube biosensor. The flash plate of these capillary tubes is not carried out with an acetone, but it was made to dry before performing a certain derivatization on a capillary tube front face.
[0111]
(Capillary tube derivatization and immobilization of a DNA probe)
The fused silica capillary tube (the bore [of 50 micrometers] x outer diameter of 150 micrometers, die length of 1m) was used for the biosensor. The capillary tube was covered with the thin layer of an epoxy resin (Epotek350) in order to cover a fused silica front face with organic covering. Surface organic covering not only makes DNA adsorption of the wall of a capillary tube the minimum, but gives the polymerization-ized front face where a DNA probe may be fixed directly. The protocol which covers a capillary tube front face with an epoxy resin was as Liu and others (Liu et al.(1996) J.Chromatogr.723:157-167) having explained correctly. It dried under the nitrogen pressure of 20psi, and when saying simply, after carrying out the rinse of the capillary tube for 15 minutes with an acetone first, it was dried in oven at 100 degrees C for 1 hour. Epoxy resin 314ND (Epo-Tek, Billerica, MA) was dynamically covered on the capillary tube front face by attracting the acetone solution of epoxy resin mixture. The residual solvent was removed from the epoxy resin covering capillary tube by carrying out a flash plate with nitrogen for 30 minutes with a room temperature. Epoxy resin covering constructed the bridge over the pan at 150 degrees C under the nitrogen pressure of 20psi(s) for 30 minutes by 80 degrees C for 2 hours. The buffer solution washed the covered capillary tube for 30 minutes before use.
[0112]
Subsequently, the flash plate of the 1cm section of an epoxy resin covering capillary tube was carried out with the specific DNA probe solution. The DNA probe solution was made to react with the piece of a capillary tube overnight, and the DNA probe was combined with the capillary tube wall by the canal

and the electrostatic interaction. Other DNA probes were fixed like the piece of a covering capillary tube of same 1cm length. When the probe was fixed by the capillary tube wall, after being deionized water and rinsing those hybridization fields, the preparation assembled to a capillary tube biosensor was completed. The distance from an inlet port to the 1st probe (TB probe) is about 25cm, and these hybridization fields were pasted up on the "separation column" with the epoxy resin in two different locations which the probe whose number is two left 15cm. Thereby, the distance of about 60cm was left behind from the 2nd probe (HIV probe) even to the detector. It connected by [both] pasting up a capillary tube on the sleeve (180x360-micrometer capillary tube section) which is die length of about 1cm respectively too about the segment from which a capillary tube differs with an epoxy resin. The overall length of a capillary tube biosensor was about 1m.

[0113]

(DNA label-hybridization, elution, and detection)

The capillary tube was attached in the capillary-electrophoresis device for commerce (Biorad Instruments Inc, Hercules, CA), and this device was used for that pressurization flow and an automatic-sampler function. The protocol used in order to hybridize a complementary target with high stringency to these DNA probes is extensively indicated by reference. The specific protocol used for this experiment is as follows.

[0114]

First, in order to make a probe and a selection target combine a cDNA target, the flash plate of the capillary tube was carried out with the pre hybridization buffer solution (inside of 0.75M NaCl, 75mM sodium citrate, a pH=7.0 or 0.1%N-lactoyl sarcosine, 0.02%SDS, and 50% formamide, 40 degrees C). The flash plate of the DNA target solution of both TB and a HIV target was dissolved and carried out to the pre hybridization buffer solution, it incubated for about 30 minutes in the capillary tube, and the perfect hybridization and the saturation of a surface fixed probe were obtained.

[0115]

Subsequently, it is the hybridization buffer solution (0.3M NaCl, 30mM sodium citrate, pH=7.0 or 0.1% SDS), and it was begun to rinse a surplus target solution. After that, in order that the DNA target as for whom any did nonspecific association might also remove, the stringent washing buffer solution (75mM NaCl, a 7.5mM sodium citrate, pH=7.0 or 0.1%SDS, 40 degrees C) performed stringent washing. Since all other things were probed under these stringent conditions by this stringent washing, by it, it was guaranteed that only a complementary DNA target is completely left behind to the interior of a capillary tube biosensor.

[0116]

Subsequently, in order to begin to rinse the high stringency washing buffer solution which does not suit a capillary tube with a copper electrode (to the existence of a surface active agent sake), it filled up with the electrochemical washing buffer solution (89mM TRIS, 89mM boric acid and 1mM EDTA, pH=10).

[0117]

When the capillary tube was filled up with the electrochemical washing buffer solution, the copper electrode was once maintained at the biosensor capillary tube outlet. The electrode was automatically aligned with the capillary tube outlet by two PERT machining design (two-part machined design) (Kuhr (1993) U.S. Pat. No. 5,650,061). Subsequently, the elution buffer solution (89mM Tris, 89mM boric acid and 1mM EDTA, pH=11) was filled quickly (with 100psi), and the capillary tube was incubated for 30 minutes at the room temperature. The elution buffer solution promoted the hybridized DNA label-denaturation, and emitted oligomer into the solution inside a capillary tube by it in the specific location.

[0118]

Subsequently, pump feeding of the elution buffer solution including the dehybridized target DNA was carried out by the fixed rate of flow using the pressurization induction flow by about 5 psi(s), and the DNA target emitted when they moved with the buffer solution by it was eluted. When DNA target oligomer passes a detector and flowed, DNA oxidized by electrocatalysis by the copper electrode, and, thereby, generated the signal which may be detected using a sinusoid voltammetry as stated above (refer to U.S. Pat. No. 5,650,061). After that, the zone according to each [of DNA] was detected by the

copper electrode of an outlet, when DNA passed a detector and moved.
[0119]
(Electrochemical detection)
Copper microelectrode with a diameter of 40 microns was manufactured inside 5cm and 50x360-micrometer fused silica capillary tube. The capillary tube was filled with the gallium using the syringe. Next, after inserting the copper wire of small die length in a capillary tube by the end, it sealed in the proper place by epoxy resin adhesion for 5 minutes. Another wire was inserted from the back end of a capillary tube, and electrical connection with a copper wire was given. The gallium inside a capillary tube gave the electrical connection between two wires. Such capillary tube microelectrodes are very strong, and reusable after polish. These electrodes were not pretreated with any gestalten except polish by the hand using the sandpaper of 600 grain size.
[0120]
The sinusoid voltammetry was used in order for copper microelectrode to detect the dehybridized DNA target, when it is eluted from a capillary tube. The protocol for performing a sinusoid voltammetry is mentioned already (Singhal et al.(1997) Anal.Chem.69:4828-4832; U.S. Pat. No. 5,650,061). When saying simply, digital generation of the sine wave of 2Hz, 0.7 ****-p, and +0.35V direct current offset was carried out using the software program in a company. This sine wave was committed as impression potential to a copper electrode. The current responses from an electrode were collected with software on real time between the single overall lengths of an elution run. Subsequently, this time amount domain current response was changed into the frequency domain by the fast Fourier transform. The protocol for analyzing frequency spectrum is mentioned already (Singhal et al.(1997) Anal.Chem.69:1662-1668). The spectrum corresponding to the analyte was obtained after background subtraction and a digital phase lock as stated above (above-shown Singhal et al. (1997)).
[0121]
(A result and consideration)
Since DNA is clinically important as an index of a disease, the amount of low of DNA hybridization and direct detection are desirable. Once it is shown that a specific nucleotide sequence is connected with a predetermined marker (for example, an infectious agent, an inherited character, a neoplasm type) characteristic or identifiable, the array is compounded in large quantities, and in order to determine whether the specific array exists, it can be used as a probe of a nucleic acid from other sources of supply. In many cases, the DNA assay based on hybridization is developed for the application from which many differ, the fingerprints of the existing DNA are carried out completely, and in order to identify, two or more trials need to be performed about all samples.
[0122]
The sinusoid voltammetry which is a frequency domain voltammetry detection technique can be used in order to detect a nucleic acid under the same experiment conditions as what is used for detection of a saccharide. A nucleotide can also be contributed to a nucleobase by those bases apart from that by which a certain signal of a nucleotide is based on a sugar principal chain since they are also electrical activities on a copper front face, including an amine part.
[0123]
Detection of unguided object-ized DNA is very desirable in order to avoid all sample handling loss and a contamination problem. From what (it can work in the amount of pico liter capacity from a nano liter) can be miniaturized easily, without sacrificing the capacity as a high sensitivity detector, electrochemical detection is suitable, especially when [of DNA analysis] a sample is restricted generally.
[0124]
In development of this capillary tube biosensor, the specific array of DNA was fixed to the field to which the interior of a continuous minute fluid channel (namely, fused silica capillary tube) differs. 1cm section of 20 bore capillary tube of 50 micrometers which is in agreement with the sample volume of nL (s) was used in order to give the recognition field of a sensor. Through each field, one by one, pump feeding is carried out, and a suitable DNA target can combine a sample with a target independently with

each fixed DNA probe there (if it exists). Once the sample had an opportunity to interact with the target by which each was fixed, elution of it was carried out from the capillary tube, the whole capillary tube was washed by a series of stringent washing, and all possibility of polluting an ingredient by it was eliminated. Subsequently, the target [having combined with each field of the fixed probe] DNA was eluted in the format by which the code was carried out spatially.

[0125]

Drawing 1 shows the fundamental approach used in this design, in order to give possibility of observing two or more hybridization events in a single experiment. Zones 1 and 2 are fixed zones where the DNA probe of TB and HIV was fixed, respectively. These zones were together put in order to produce a capillary tube system single in order to use one impregnation of the sample containing a DNA target behind. A reagent required in order to wash a more complicated sample (namely, clinical sample containing other biomolecules of a large number, such as protein and other cell strains) with very high stringency can be introduced by the flow by which pressurization induction was carried out from the reservoir of the head of a capillary tube. Copper microelectrode is arranged at the outlet edge of a capillary tube, and it is arranged using the machining two PERT system which enables automatic alignment of a capillary tube with an electrode (Kuhr's and others U.S. Pat. No. 5,650,061). Therefore, a system is very easy to combine, and once it works, it is dogged.

[0126]

The sequence of the process used in order to perform specific hybridization, washing, and elution of a target oligonucleotide that denaturalized is shown in drawing 2 . The same process can be used for all the DNA label kinds to those complementary probes of stringent hybridization. In this scheme,

1) Hybridization is performed under stringent conditions, in order to avoid all nonspecific label association to the probe which is not perfect phase complement to a capillary tube wall or the target analyte. Consequently, TB target (the oligomer (zone 1) which has an array characteristic of DNA which carries out the code of the TB is only hybridized to fixed TB probe (complementary sequence), and a HIV target only hybridizes it to the HIV probe (zone 2) fixed under stringent conditions.) These zones are isolated spatially and stringent washing removes all interferent components also from the capillary tube which separates those zones only from each zone.

[0127]

2) The last washing by the elution buffer solution (TBE, pH=11) denatures the hybridized complementary nucleic acid to coincidence, and emits the DNA target which joined together by it to the solution which adjoins the fixed probe of a capillary tube directly. Such two label spatial selectivity is maintained. It is because the buffer solution moves to a proper place quickly (with time scale also with the much high-speed twist which dehybridization may produce), and the flow in a capillary tube stops and a denaturation process is completed after the incubation for 30 minutes.

[0128]

3) Finally elution of the solution containing the "free" target DNA oligomer separated spatially is carried out. Since the zone including the two targets is spatially separate, they pass over the copper electrode arranged at the outlet to different time amount, and flow to it. The scheme shown in drawing 3 has illustrated the aspect of affairs of detecting the eluted DNA target. Each label elution time amount in a detector shows the true character, and, thereby, codes the part of DNA hybridization.

[0129]

Detection of the HIV target DNA using the capillary tube biosensor by 1cm zone of a fixed DNA probe is shown in drawing 4 . The flash plate of the sample containing 10microg [/ml] 100 synthetic HIV labelmicroL was carried out through the inside of the capillary tube biosensor with which the HIV probe was fixed. In order to enable HIV oligonucleotide label-detection of a sample, it followed in order of the process indicated to drawing 2 . Originally, the sequence did not contain the electrochemical washing buffer solution (89mM TRIS, 89mM boric acid and 1mM EDTA, pH=10). It added in order to make into the minimum the artifact observed when the elution buffer solution attacks this to a copper electrode. pH of this buffer solution is important. While too high pH leads to Target's DNA dehybridization and loss of a signal is brought about, it is because too low pH produces the big artifact

as a result when the elution buffer solution reaches a detector.
[0130]
As shown in drawing 4 , DNA label-elution is proved [signal / which was acquired with the sinusoid voltammetry] after the dehybridization in the elution buffer solution. Although it is shown that the elution of a blank solution has the very stable signal, it is difficult to evaluate the singularity of HIV label-association by the single probe system. Therefore, this kind of detection may bring about false positivity in a DNA trial.
[0131]
In the design of the proper, two or more probes system not only can tackle the problem of parallel processing of a nucleic-acid sample, but gives the internal reference over nonspecific hybridization. It will give two or more peaks in two or more probes system, when nonspecific hybridization occurs with a given sample. This will show the need for a much more stringent hybridization protocol directly until a peak single about the single target which poured in is detected. The singularity of the hybridization of this system is illustrated to drawing 5 (A), and detection of the specific label-hybridization of TB and HIV exists in coincidence in the same sample. Although the interaction of the sample was carried out only once to each DNA probe, two targets can detect to coincidence by one run. The transit time about two zones agrees with TB and the HIV label-internal reference which were shown in drawing 5 (B) and 5 (C), respectively. Therefore, this also shows that what kind of nonspecific hybridization which two targets not only can detect to coincidence, but occurs under the hybridization conditions currently used does not exist. Otherwise, probably, the internal reference run showed not one but two peaks (that is, even if TB specific target probably hybridized to self completely complementary probe and HIV specific probe and sticks in HIV specific label, he is the same). Therefore, detection of two peaks in drawing 5 (A) shows composite TB and HIV specific label-detection clearly to coincidence, has illustrated the absence of nonspecific hybridization, and reduces the hope of generating as a result of all false positivity.
[0132]
DNA sequencing by hybridization is dependent on the molecular recognition given by the hybridization to the fixed probe DNA of a sample (for example, target) DNA molecule. Die length is about 7 nucleotide at least, die length is about 10 nucleotide at least more preferably, die length is the nucleotide of 15 or 20 at least still more preferably, and the die length of a desirable probe oligonucleotide is the nucleotide of 30, 40, or 50 at least most preferably. This probe has a complementary known array to at least 1 label field. Although the assay format that a large number differ exists, after a probe contacts a nitrocellulose, agarose, plastics, or a sample, is placed and washes un-recognizing [DNA] finely, it is typically fixed by other quality of a deactivating group which can carry out assay about a content. the assay of hybridized DNA is executable in the system indicated in this detail letter with the elution from denaturation, capillary tube, or channel of DNA, and detection by SV in copper microelectrode.
[0133]
(Conclusion)
The new DNA biosensor of the capillary tube base was developed using the direct electrochemical detection which can detect two or more DNA oligomers to coincidence. This detection scheme used the DNA label flow coding hybridization assay in a sample by various DNA probes fixed by the location where capillary tube front faces are various. It is supplemented with the DNA label coincidence hybridization of various types by those label-direct detection in the copper electrode by using a sinusoid voltammetry when they elute. a disease -- such detection of a specific oligonucleotide array-like in parallel and raw is dogged, and it is durable and it can open the path to a cheap two or more disease DNA sensor. therefore, it -- activation -- an operator -- the problem accompanying the existing DNA sensor based on intensive and expensive, various optical detection schemes is avoided.
[0134]
(Example 2)
(High sensitivity of the amino acid by the sinusoid voltammetry, and a peptide, and alternative detection)

(Experiment parameter)
(Reagent)
After deionizing the water to be used, it passed the Milli-Q water purification system (Millipore Corp., Bedford, Mass.). Amino acid, the insulin (98 - 99%, and Sigma Chemical Corp. and St. Louis, Mo.), and the remaining peptide (Peninsula Laboratories, Inc., San Carlos, CA) were used received. All experiments were conducted by 0.10 sodium hydroxides (A. a C.S grade, Fisher Scientific, Fair Lawn NJ) as a migration electrolyte. The undiluted solution of 0.10M was prepared in deionized water. Future dilution was performed using the migration electrolyte.
[0135]
(Copper microelectrode)
Copper microelectrode was produced by pulling a glass capillary tube by microelectrode Pullar (Model PE-2, Narishige, Tokyo Japan) first. Then, the edge of a capillary tube was cut off by Scalpel under the microscope. Then, copper wire (99.99%, Goodfellow, Cambridge, England) with a diameter of 20 micrometers was inserted in the edge from which it was cut out newly, and was sealed with the epoxy resin (Epoxy Technology, Billerica, Massachusetts). The electrode was ground by the diamond grinding wheel and carried out clarification by sonication by deionized water. In order to make electrical connection with copper wire, the back end of a capillary tube was filled up with the gallium (Sigma Chemical Co.), and diameter the copper wire of 150 micrometers was inserted in the gallium. As an alternative, the back end of a capillary tube was filled up with the epoxy resin, and more, the copper wire of a major diameter was put into the epoxy resin restoration capillary tube until it contacted 20-micrometer wire physically. Any electrochemical pretreatments are not performed, but the electrode was stabilized until the stable response of about 1 hour was observed under experiment conditions.
[0136]
(Electrochemical measurement and experiment conditions)
The flow cell was constituted from PUREKISHI glass, and tubing was adjusted so that diffusion-breadth might be avoided. Installation of a sample plug was controlled by the air operated actuator controlled by the solenoid valve. The rate of flow was maintained by the gravity flow by maintaining a buffer-solution reservoir on 19cm of a flow cell. It determined that the rate of flow was a part for 0.5ml/, and the volume of a sample was determined from the rate of flow and die length of impregnation. Impregnation time amount determined that an electrode will look at the perfect concentration of the analyte.
[0137]
The reported conditions of an experiment are explained here. In the case of amino acid and a peptide, 2Hz sine wave (0-690mV pair Ag/AgCl) applied with the software written by the author by Labview (National Instruments, Austin, Tex.). Wave filtration of the wave was carried out with the 4 super-low region filtration filter using cyberamp (Model 380, Axon Instruments Inc., Foster City, CA.) with 3db point of being 3 times (6Hz) many as fundamental frequency. Wave filtration of the output current was carried out with the 4 super-low region passage filter. The filter was set as 40Hz (4 times, the 10th higher harmonic, or 20Hz of the observed maximum frequency). A current is 300MHz. Pentium (trademark) It changed into the analog from digital ones by the 16-bit analog-to-digital converter (PCI-4451, National Instruments) using II personal computer. The single scan consisted of 4 sine-wave periods.
[0138]
With Labview software (National Instruments), the collected time amount domain was changed into the frequency domain, and was further processed using Matlab programming (The Mathworks, Inc., Englewood Cliffs NJ). The spectrum of only a signal was obtained by lengthening the background vector acquired before impregnation from an instant signal current vector. In order to acquire a time amount domain spectrum, the digital lock in amplifying method was used. In order to generate the amplitude of each frequency higher harmonic (up to the 10th higher harmonic of max), and a phase angle, the Fourier transform of the time amount spectrum was carried out at the rate of 512 points. The vector of only a signal was used for the topology of each higher harmonic wave, and it acquired it by projecting it on a background subtraction signal vector. Finally, moving-average smoothing (cube type

integral) was used for the phase decomposition vector, and it carried out low-pass wave filtration passage.

[0139]

(Result)

Drawing 6 shows the background subtraction frequency spectrum of the arginine in copper microelectrode. The experiment was performed using 1microM arginine. The excitation signal was the sine wave of 2Hz and 0-690mV pair Ag/AgCl. The current from 4 sine wave periods which consist of 512 points (whole time amount = 1 second) was used in order to generate each frequency spectrum. The three-dimensions graph consists of the frequency (x axis), the amplitude (z-axis), and phase angle (y-axis) information to the 10th higher harmonic.

[0140]

Drawing 7 shows the sinusoidal time amount domain response from 1microM arginine in the 5th higher harmonic (10Hz). This higher harmonic gave the highest signal / noise ratio, and the limit of detection (S/N=3) of 39nM(s).

[0141]

Drawing 8 shows the linearity dynamic range of various arginine concentration. The arginine concentration of 1, 10,100, and 1000microM was poured into the flow impregnation analysis system. The amplitude of the 5th higher harmonic (10Hz) is plotted to four poured-in different concentration. This plot shows the outstanding linearity (R= 0.9997) covering 3 order in the 5th higher harmonic.

[0142]

Drawing 9 shows the asparagine in copper microelectrode, and the subtraction frequency spectrum of a glutamine. A square expresses 10microM asparagine and the circle expresses 10microM glutamine. Experiment conditions are the same as what was used in order to generate drawing 1 .

[0143]

Drawing 10 A and 10B show the sinusoidal time amount domain response of the asparagine in the 6th higher harmonic (12Hz), and a glutamine. Drawing 10 A shows 10microM asparagine, and drawing 10 B shows 10microM glutamine. The 6th higher harmonic has the optimization phase angle of those two amino acid closest to 90-degree separation. This higher harmonic gives the maximum selectivity in between those two analyte. In the case of an asparagine, the limit of detection (S/N=3) in this higher harmonic is 400nM(s), and, in the case of a glutamine, is 500nM.

[0144]

Drawing 11 shows the background subtraction frequency domain spectrum of 10microM insulin B chain. The same conditions as drawing 1 were used.

[0145]

Drawing 12 shows the sinusoidal time amount domain component of the insulin B chain in the 4th higher harmonic (8Hz). The 4th higher harmonic gave the greatest signal / noise ratio, and the limit of detection (S/N=3) of 500nM(s).

[0146]

Drawing 13 shows the luteinizing hormone releasing hormone (circle) in copper microelectrode, and the subtraction frequency spectrum of bradykinin (square).

[0147]

Drawing 14 A and 14B show the time amount domain response of the bradykinin in the 2nd higher harmonic wave (4Hz), and luteinizing hormone releasing hormone, respectively.

[0148]

Drawing 15 shows the background subtraction frequency domain response of neurotensin (square) and substance P (circle), respectively.

[0149]

Drawing 16 A and 16B show the time amount domain response of the neurotensin in fundamental frequency (2Hz), and substance P, respectively.

[0150]

The example and operation gestalt which were explained here are for for the purpose of instantiation, in

the light of it, various corrections or modification are submitted to this contractor, and it is understood that it should be contained in the inside of the pneuma of this application, the text, and an attachment claim. Therefore, all the publications quoted here, a patent, and patent application are taken in by reference in a perfect form by all the purpose here.

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1]
Drawing 1 shows the schematic drawing of the DNA biosensor of the capillary tube base by electrochemical detection. Two different probe sections exist in a capillary tube. They are the probe 1 of TB specific probe, and the probe 2 of a HIV specific probe. A HPCE automatic sampler is used for various stringent washing and rinses required for the cDNA label specific hybridization of these fixed probes. A copper electrode is arranged at the outlet of a capillary tube biosensor using a machining two PERT system.
[Drawing 2]
Drawing 2 shows the protocol for performing DNA label stringent hybridization and alkali denaturation inside a capillary tube biosensor. (1) Hybridize various DNA targets to the probe fixed by the capillary tube front face. (2) After that, stringent washing is performed in order to remove one of nonspecific adsorption or DNA which were hybridized. (3) Alkali denaturation is performed by ** which finally elutes the DNA target which hybridized from the capillary tube biosensor before.
[Drawing 3]
Drawing 3 shows the elution from a DNA label capillary tube biosensor by which alkali denaturation was carried out, and the continuing schematic drawing of electrochemical detection. An electrode is manufactured inside the piece of a capillary tube equipped with the same diameter as a biosensor capillary tube, in order to make automatic alignment easy. An electrode will carry out a location extremely at the outlet of a biosensor capillary tube soon (<5micrometer). Lower trace shows the schematic drawing of the DNA label-detection at the time of their eluting from a biosensor capillary tube.
[Drawing 4]
Drawing 4 illustrates the HIV specific label-detection which used a capillary tube biosensor and sinusoid voltammetry detection. A 10microg [/ml] HIV specific target is passed inside the capillary tube biosensor with which only the HIV specific probe was fixed. All hybridization conditions are as a publication in this specification. The sinusoid voltammetry excitation wave was 2Hz in 0 - 700 mVp-p. The illustrated signal was acquired by the 5th higher harmonic.
[Drawing 5]
Drawing 5 shows two or more DNA label-detection which used flow coding hybridization assay for coincidence. The used sample contained 1:1 mixture of concentration with a specific label-each [of HIV and TB] of 10microg [/ml]. All hybridization and elution conditions are the same as what was explained in the thing and example 1 in drawing 4 . Since detection understood the illustrated signal that it has the best sensibility, it was acquired by the 5th higher harmonic.
[Drawing 6]
Drawing 6 shows the background subtraction frequency spectrum of the arginine in copper microelectrode. The three-dimensions graph consists of the frequency (x axis), the amplitude (z-axis), and phase angle (y-axis) information to the 10th higher harmonic.

[Drawing 7]
Drawing 7 shows the sinusoidal time amount domain response from 1microM arginine in the 5th higher harmonic (10Hz).
[Drawing 8]
Drawing 8 shows the linearity dynamic range of various arginine concentration.
[Drawing 9]
Drawing 9 shows the asparagine in copper microelectrode, and the subtraction frequency spectrum of a glutamine. A square expresses 10microM asparagine and the circle expresses 10microM glutamine.
[Drawing 10]
Drawing 10 A and 10B show the sinusoidal time amount domain response of the asparagine in the 6th higher harmonic (12Hz), and a glutamine. Drawing 10 A shows 10microM asparagine, and drawing 10 B shows 10microM glutamine.
[Drawing 11]
Drawing 11 shows the background subtraction frequency domain spectrum of 10microM insulin B chain.
[Drawing 12]
Drawing 12 shows the sinusoidal time amount domain component of the insulin B chain in the 4th higher harmonic (8Hz).
[Drawing 13]
Drawing 13 shows the luteinizing hormone releasing hormone (circle) in copper microelectrode, and the subtraction frequency spectrum of bradykinin (square).
[Drawing 14]
Drawing 14 A and 14B show the time amount domain response of the bradykinin in the 2nd higher harmonic wave (4Hz), and luteinizing hormone releasing hormone, respectively.
[Drawing 15]
Drawing 15 shows the background subtraction frequency domain response of neurotensin (square) and substance P (circle), respectively.
[Drawing 16]
Drawing 16 A and 16B show the time amount domain response of the neurotensin in fundamental frequency (2Hz), and substance P, respectively.

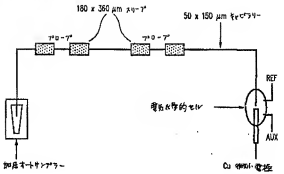
[Translation done.]

* NOTICES *

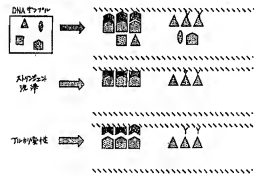
1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DRAWINGS

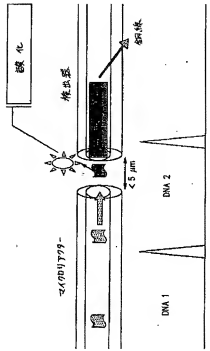
[Drawing 1]



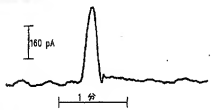
[Drawing 2]



[Drawing 3]

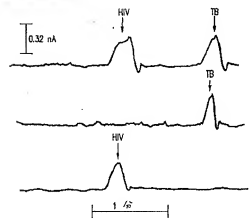


[Drawing 4]

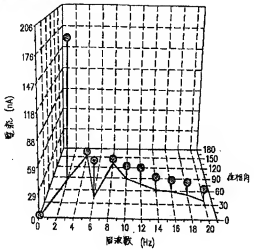


固定されたＴＢ＋ＨＩＶプローブを有するキャピラリーＤＮＡバイオセンサー。
各プローブ領域の長さ＝１ｃｍ。収域間の距離＝７インチ。
３０分のインキュベーションの後、ＴＢＥ緩衝液（ｐＨ＝１１）で標的を溶出。
検出：４０μｍ直径を用いるシヌソイドボルタンメトリー
２Ｈｚ，Ｅ＝７００ｍＶｖｓ正電流。第６高調波で示された応答。

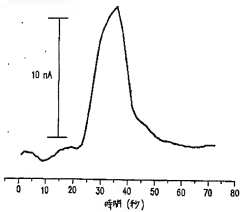
[Drawing 5]



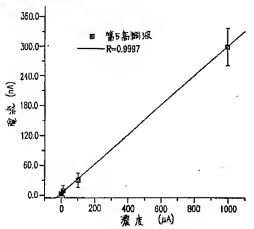
[Drawing 6]



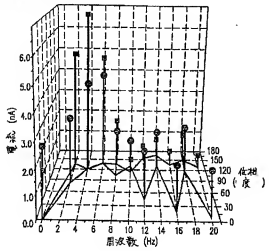
[Drawing 7]



[Drawing 8]



[Drawing 9]



[Drawing 10]

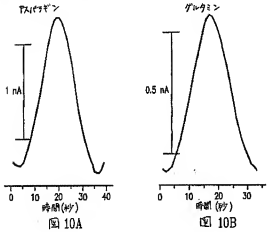
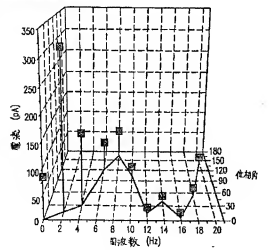
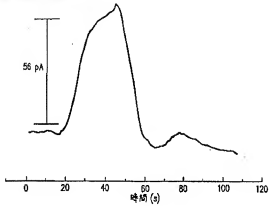


図 10A 図 10B

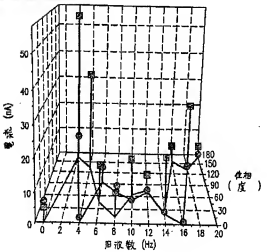
[Drawing 11]



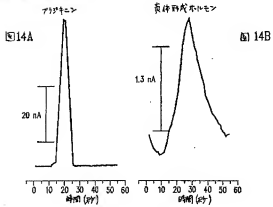
[Drawing 12]



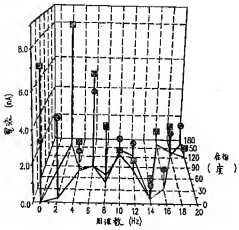
[Drawing 13]



[Drawing 14]



[Drawing 15]



[Drawing 16]

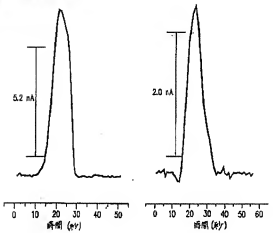


図 16A 図 16B

[Translation done.]